An in-depth investigation into the associations between maternal dietary intake during pregnancy and obesity and allergy outcomes in children

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Abstract

Maternal diet in pregnancy and early infant diet have been implicated in the aetiology of obesity and allergy. This thesis aims to investigate the effect of maternal nutrition during pregnancy and infant feeding practices in early life on the development of obesity and allergic outcomes in children.

This research was conducted as two separate complementary studies. The first synthesised the best available evidence from randomised clinical trials, by conducting two systematic reviews, of the effectiveness of maternal nutritional/dietary interventions during pregnancy to prevent obesity and allergic outcomes in offspring. The second collected data prospectively from the Portsmouth Birth Cohort registry on maternal diet during pregnancy as well as feeding practices of babies at 2 and 6 months of age, and assessed how these nutritional behaviours affected the development of weight and allergic outcomes in babies by 6 months of age.

The systematic reviews provided evidence that prenatal supplementation of probiotics, fatty acids and vitamins could protect against childhood eczema, sensitisation and wheeze respectively. However, nutritional/dietary interventions did not prevent obesity in children. The second study showed three main findings: 1) higher maternal consumption of sugar during pregnancy was associated with lower weight Z-score both at birth and at 6 months of age; 2) partially breast-fed babies compared to dominantly breast-fed and formula-fed babies at 2 months had lower weight Z-score at 2 and 6 months of age; 3) the introduction of wheat at 3-6 months compared to later introduction was associated with fewer allergic symptoms at 6 months of age.

The novel findings of this research have implications for practice. Notably, Vitamin D intake in pregnancy was found to prevent wheeze in children; however, longer-term follow-ups of these studies is necessary to determine whether Vitamin D could also protect against childhood asthma. Findings of the cohort highlight the importance of healthy diet in pregnant women and early feeding practices in babies for the development of obesity and allergies in babies. Longer-term follow-up of these babies in a larger sample is needed to validate these results.
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Declaration

Whilst registered as a candidate for the above degree, I have not been registered for any other research award. The results and conclusion embodied in this thesis are the work of the named candidate and have not been submitted for any other academic award.

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Abbreviation list

AA= Arachidonic Acid
AD= Atopic Dermatitis
AHEI-P= Alternative Healthy Eating Index in Pregnancy
AHEI= Alternative Healthy Eating Index
AHRR= Aryl-Hydrocarbon Receptor Repressor
ARC= Allergic Rhino-Conjunctivitis
ARIA= the Allergic Rhinitis and its Impact on Asthma
BMI= Body Mass Index
BOS= Bristol Online Surveys
CDC= Centers for Disease Control and Prevention
CDSR= Cochrane Database of Systematic Reviews
CHILD= The Canadian Healthy Infant Longitudinal Development
CI= Confidence Intervals
CMP= Cow’s Milk Protein
COAST= the Childhood Origins of Asthma study
CVDs= Cardiovascular Diseases
DARE= Database of Reviews of Effectiveness
DBPCFC= Double-Blind Placebo-Controlled Food Challenge
Der p= Dermatophagoides pteronyssinus
DHA= Docosahexanoic acid
DOHaD= Developmental Origins of Health and Disease
DPA= Dococapentaenoic Acid
EAACI= European Academy of Allergology and Clinical Immunology
EPA= Eicosapentaenoic Acid
ETHoS= E-theses Online Services
Evidence-based practice
FEV1= Forced Expiratory Volume in 1-second
FFQ-P= Food Frequency Questionnaire in Pregnancy
FFQ= Food Frequency Questionnaire
GDM= Gestational Diabetes Mellitus
GWAS= Genome-Wide Association Studies
GWG= Gestational Weight Gain
H2RAs= Histamine 2-Receptor Antagonists
ICD= International Classification of Diseases
ICTRP= International Clinical Trials Registry Platform
IgE-mediated= Mediated by Immunoglobulin E
IL= Interleukin
IOM= Institute of Medicine
IOTF= International Obesity Task Force
IPAQ= International Physical Activity Questionnaire
ISAAC= International Study of Asthma and Allergies in Childhood
ITT= Intention to Treat Analysis
LEAP= Learning Early About Peanut Allergy
LGA= Large for Gestational Age
LG index= Low Glycaemic index
MD= Milk Diary
NCDs= Non-communicable Diseases
NHS= National Health Institute
NIAID= National Institute of Allergy and Infectious Disease
ONS= Office for National Statistics
PASTURE= the Protection against Allergy: Study in Rural Environments
PATCH= Prediction of Allergies in Taiwanese Children
PATY= Pollution and the Yung study
PBC= Portsmouth Birth Cohort
PNMS= Pre-Natal Maternal Stress
PPIs= Proton Pump Inhibitors
PRISMA= Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PUFAs= Long-Chain Poly-Unsaturated Fatty Acids
QA= Queen Alexandra (Hospital)
R&D= Research and Development
RCTs= Randomised Controlled Trials
ROB= Risk of Bias
RR= Relative Risk/Risk Ratio
SACN= the Scientific Advisory Committee on Nutrition
SCORAD= Scoring Atopic Dermatitis
SD= Standard Deviation
SFT= Skin Fold Thickness
SGA= Small for Gestational Age
sIgE= specific Immunoglobulin E
SMD= Standardised Mean Difference
SOC= Standard Occupational Classification
SPSS= The Statistical Package for Social Sciences
SPT= Skin Prick Test
UK= United Kingdom
US= United States
$\chi^2$= Chi$^2$ Test
WAO= World Allergy Organisation
WHO= World Health Organisation
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Dissemination

Journal publications


Poster presentation


- **Vahdaninia, M.**, Mackenzie, M., Helps, S., Dean, T. Fatty acid supplementation during pregnancy and allergic outcomes in the offspring: a systematic review and meta-analysis. Developmental Origins of Health and Disease (Canadian meeting), Alberta (Banff) 2018

- **Vahdaninia, M.**, Mackenzie, M., Helps, S., Dean, T. The effectiveness of maternal fatty acid interventions during pregnancy on obesity in offspring: a systematic review and meta-analysis. Developmental Origins of Health and Disease (Canadian meeting), Alberta (Banff) 2018

Oral presentation

Chapter 1: General introduction

1.1. Background

The first 1,000 days of life, the period between a woman’s pregnancy and her baby’s second birthday is key to good health for a life-time. The new developmental model states the origin of many chronic diseases including metabolic disorders/obesity and allergies stem from this period (Barker, 2012), thus the very early days in an infant’s life provides a unique opportunity to build and improve the health of future generations. In this context, the role of environmental factors such as diet and lifestyle-related behaviours are key for primary/early prevention of chronic diseases (Mayor et al., 2015; Thornburg & Marshall, 2015). In fact, the foetus starts responding to the external stimuli when in utero and hence, factors such as maternal diet could directly programme an infant’s immune system without necessarily affecting birth size (Campbell et al., 1996; Jansson & Powell, 2007; Ravelli et al., 1998; Roseboom et al., 2001; Shiell et al., 2001). Women in the Western world predominantly follow an inadequate and unbalanced diet while many women in developing countries suffer from malnutrition; both circumstances could have long-term impact on the health of their babies (Barker, 2012). On this basis, interventions aiming to improve nutritional adequacy in pregnant women could considerably contribute towards reducing the incidence of chronic diseases.

The influence of nutrition on the development of a baby is also prominent after birth when the infant comes into contact with the postnatal environment via breast or bottle-feeding and later through weaning and introduction of other foods. It is well documented that maternal diet and the composition of breast milk have immunomodulatory effects on the infant’s immune system and consequently could initiate the development of chronic diseases later in life (Munblit et al., 2017; Victora et al., 2016).

An overwhelming body of evidence from experimental and epidemiological studies suggests that poor early life environment, stimulated by both quantity and quality of nutrients during the critical phases of development i.e. in utero and early postnatal life can make evident changes in the developing foetus (Bertram & Hanson, 2001;
Armitage et al., 2004; Barker 1990; Langley-Evans, Phillips & Jackson, 1994; Whincup et al., 2008). In other words, either under or over-nutrition during both gestation and postnatal life could induce an adaptive response to environmental signals in the developing foetus/child, which allows its developmental growth to be modified towards nutritional thifts for future environmental insults (Gluckman & Hanson 2004; Wells 2009). These effects, termed as programming (Lucas 1991), establish permanent physiological and metabolic states in the foetus, and could potentially increase the risk of a range of long-term health conditions in adulthood, including non-communicable diseases (NCD) (Lillycrop 2011; Langley-Evans 2015).

Large population studies on babies exposed to large historical famines have shed more light on the links between nutritional balance during early stages of life and later risk of diseases. It has been shown that babies born during or immediately after these incidents had an elevated risk of type-2 diabetes mellitus, hypertension and metabolic syndrome (Li et al., 2011; Schulz, 2010; Thurner et al., 2013).

The mechanisms through which the nutritional challenges in early life could introduce lasting changes in the foetus, which could link to its future health, are not fully understood. Current knowledge proposes that intrauterine nutrient availability can alter the epigenome, the chemical compound that can instruct DNA, but the sequence in DNA (Doherty, Mann, Tremblay, Bartolomei, Schultz 2000; Khosla, Dean, Reik, Feil 2001). These epigenetic marks in the regulation of imprinted genes cause heritable alterations to the phenotype of the offspring (Swali, McMullen, Hayes, Gambling, McArdle & Langley-Evans 2011; Swali, McMullen, Hayes, Gambling, McArdle & Langley-Evans 2012). Two main factors of epigenetic influences are DNA methylation and histone modifications where DNA methylation delivers mechanism for gene silencing and the histon modification either allow gene transcription or silence expression (Jaenisch & Bird, 2003; Burdge, Hanson, Slater-Jefferies, & Lillycrop 2007). Nutritional imbalances, excess or deficit of nutrients, in early life stage are most likely to affect DNA methylation as evidence from the Dutch famine study showed methylation differences at the Insulin-like Growth Factor 2 (IGF2) locus between individuals exposed to famine and their unexposed siblings (Heijmans et al., 2008). Despite recent advances in this area, there is still a need for greater understanding of mechanisms which could explain the role of early nutritional status and development of epigenetic markers and thus phenotype and later disease
risk (Lillycrop & Burdge 2015). It is also necessary to add that the existing knowledge is largely reliant on experimental studies which should be replicated in human studies because of the structural and physiological differences.

Given the importance of nutrition and its influential role on developing chronic diseases in today’s world, the United Nations has declared 2016-2025 as the decade of nutrition (World Health Organization, 2017). This calls for a change in how we define the quality of nutrition since globally malnutrition in all its forms (undernutrition, micronutrient deficiency, overweight/obesity) affects a large proportion of people, while a balanced rich diet from the earliest days in life could be the solution to good health in the next generation(s). This thesis will examine the effect of maternal nutrition in pregnancy, in two systematic reviews of interventional studies and also in an original prospective birth cohort, and feeding practices early in life and their potential as risk factors for childhood allergies and obesity.

1.2. Aims and research questions

The aim of this research programme was to assess whether maternal diet during pregnancy and early infant feeding could affect the development of obesity and allergic outcomes in their offspring. Two separate complementary studies were conducted to address this aim.

Study 1 (systematic reviews and meta-analyses):

The following research questions were examined in this first study:

- Are nutritional/dietary interventions during pregnancy effective in preventing allergic disorders in offspring?
- Are nutritional/dietary interventions during pregnancy effective in preventing obesity in offspring?

Study 2 (prospective observational study in the city of Portsmouth of pregnant women and their children at 2 and 6 months age follow-ups):

The following research questions were examined in this second study:

- Does quality of maternal diet during pregnancy affect weight/birth Z-score at 6 months of age?
Does quality of maternal diet during pregnancy affect incidence of allergies at 6 months of age?

Do infant feeding practices at 2 months of age affect weight/Z-scores at 2 and 6 months?

Do infant dietary behaviours at 6 months of age affect the development of allergies at 6 months?

Of note, the city of Portsmouth, as one of the deprived cities in the UK, has a health status below the national average and there are significant health inequalities (Hirsch & Valadez, 2014). In this context, children and young people are particularly disadvantaged and more targeted interventions are required. The results of the observational study, as the first cohort in the city with the potential for longitudinal follow-ups, can provide valuable data that are locally relevant and informative at a national level.

1.3. Rationale of the study design

Evidence-based practice (EBP) is nowadays the cornerstone of clinical practice and its importance in day-to-day practice has long been recognised. The term EBP was first described in 1996 by Professor David Sackett as “the conscientious, explicit and judicious use of current best evidence in making decisions about the care of the individual patients. The practice of EBP means integrating individual clinical expertise with the best available external clinical evidence from systematic research” (Sackett, Rosenberg, Muir Gray, Haynes, Richardson 1996). From a clinical point of view, EBP values the clinical expertise of clinicians while also taking into account patients’ values in combination with the best research evidence. Effective EBP demands time and involves five essential steps (Sackett, et al., 1997; McKibbon 1998). Following formulating an answerable research question (1st), the available evidence needs to be retrieved from valid resources (2nd) which subsequently leads to the appraisal of the evidence in order to make a sound decision (3rd). Further steps comprise applying the decision and then evaluation/analysing the outcome obtained from using the evidence respectively. In the above EBP cycle, formulating a good clear research question is a key step that helps to efficiently search for the previous work done and also, to identify the appropriate type of study design.
In the hierarchy of evidence, meta-analyses and systematic reviews provide the strongest evidence since they answer a precisely formulated research question applying rigorous systematic methods to identify, appraise, collect and analyse data from the available and selected literature. A particular benefit of doing a systematic review is that it brings together a number of separately conducted studies, with different quality and sometimes contrasting findings, and synthesises their results to produce summaries of the evidence (Cook, Mulrow, Haynes 1997). Meta-analyses can be conducted if there is a possibility to unite data from individual studies and re-analyse using established statistical methods.

Systematic reviews, if well conducted, could provide reliable objective answers to a specific clinical question and also, identify the gap(s) in the current evidence. Likewise, it can be decided how their findings are generalisable to populations, settings and treatment variations and therefore, could help health practitioners to make informed decision-makings.

Randomised controlled trials (RCTs), at the 2nd level of hierarchy of evidence, use an experimental design in order to test the effectiveness of a new treatment, diagnosis or screening method. A basic trial design follows a PICO format: population of interest (P), the intervention(s) (I), comparison/control defined as placebo/standard treatment

Figure 1.1. Hierarchy of evidence in the study design (adapted from Melnyk 2011)
Given the importance of systematic reviews in clinical decision-making, the first study in this PhD project aimed to synthesis the best available evidence from RCTs, a highly ranked evidence-based study, on the effectiveness of nutritional interventions in pregnant women for the prevention of allergies and obesity outcomes, as the two most common NCDs in children. In the second study, a cohort approach was used, as the highest ranked evidence-based observational study design, to investigate how is the dietary intake of pregnant women in Portsmouth and how this intake was related to allergies and growth outcomes in their babies. Feeding practices of babies were also collected at 2 and 6 months in a prospective approach. The cohort study was best choice for the second study since women could neither have been randomised to follow a specific diet nor to breast or bottle feed their babies.

1.4. Thesis layout

The opening chapter of this thesis, chapter two reviews the relevant literature on early origins of allergies and obesity, including the literature specific to individual chapters of this thesis. Following an introduction to the Developmental Origins of Health and Disease (DOHaD) theory (Barker, 2012), the literature review continues with a detailed background on definition, prevalence and diagnosis of allergies and obesity; it then outlines the primary risk factors, in utero and postnatal milieu for these conditions. The chapter ends with a discussion around reasoning on prenatal nutritional interventions for the primary prevention of childhood allergies and obesity. Of note, the literature review is outlined separately for allergic and obesity outcomes.

Chapter three presents the synthesis of evidence from RCTs on the effectiveness of maternal dietary interventions (pro/prebiotics, fatty acids, food avoidance
interventions, vitamins/supplements) during pregnancy for the prevention of allergic diseases in offspring. The chapter outlines the methodology for the conduct of systematic reviews and subsequently, the results and risk of bias (ROB) assessments are separately presented for each intervention group.

Chapter four describes the synthesis of evidence from RCTs on the effectiveness of maternal dietary interventions (fatty acids, pro/prebiotics, low glycemic index, lifestyle change, vitamins/supplements) during pregnancy for the prevention of obesity in offspring. It follows a similar outline to that of chapter three.

Chapter five describes the first cohort study on the quality of maternal diet and infant feeding practices on weight/age Z-scores. Participants for this study were pregnant women from the Portsmouth birth cohort registry and their children who were followed up at 2 and 6 months of age. The associations between the defined risk factors and outcomes are investigated in linear regression models.

Chapter six reports the effects of the quality of maternal diet and infant dietary behaviours at 6 months of age on developing allergies in the same cohort. Statistical analyses were conducted for individual risk factors and the influential effect of all factors were examined in a logistic regression model.

Chapter seven describes the overall findings from the conducted systematic reviews and meta-analyses and discusses the results in the context of current literature. The findings from the cohort studies are also collated and discussed in view of existing literature. The implications from the conducted studies are outlined and future research needs along with the strengths and limitations of the research addressed.
Chapter 2: Literature review

2.1. Overview of the chapter

This literature review intends to give an overview of the early origins of allergies and obesity. It first explores the concept of the Developmental Origins of Health and Disease (DOHaD) theory and then reviews the current evidence in the area of childhood allergy and obesity. It describes the definition, prevalence and diagnosis of allergic disorders and obesity and the risk factors for these conditions both in utero and in the postnatal environment as well as early prevention strategies focusing on prenatal nutrition interventions.

2.2. Developmental Origins of Health and Disease theory

The DOHaD theory focuses on the key sensitive stages of human development defined as plasticity course and provides a novel model for early prevention of common chronic diseases (Barker, 1997; Gillman, 2005). This theory challenges the traditional attitude to the origins of disease as it proposes that the development period is not dictated by a hard-wired genetic programme; instead the organism responds to the surrounding environment and so the risk of many diseases is set during this time (Bernal Autumn, 2010). Subsequent to the substantial increase in the incidence of many Non-communicable Diseases (NCDs) during the last 20-40 years, it has been proposed that there is an important role for environmental factors in the onset of these complex conditions and that the role of fixed genetic variation is far less than previously believed (Barouki, Gluckman, Grandjean, Hanson, & Heindel, 2012). In other words, while genes may predispose individuals to develop these diseases, these conditions develop only if relevant environmental and lifestyle factors occur. NCDs, also known as chronic diseases, are diseases that are not passed from one person to another, typically characterised with a long duration and slow progression. Main types of NCDs are Cardiovascular Diseases (CVDs), which claim 17.3 million deaths a year, followed by cancers, chronic respiratory diseases and asthma, diabetes (Lim et al., 2012) as well as allergic diseases. NCDs are characterised by chronic, low-grade inflammation, highlighting the involvement of the immune system, a common feature to allergic diseases. NCDs and allergy also share common life-style related and environmental risk factors including modern dietary patterns and environmental
pollutants (Prescott, 2014). Therefore allergies are considered in the context of NCDs as the most common and early-onset NCD. NCDs pose an enormous global burden and account for 36 million deaths per year (World Health Organisation, 2010), where childhood obesity is linked with a raised level of premature death in adulthood (The World Health Organisation, 2014). The most recent systematic analysis for the Global Burden of Disease reported a continued rise of NCD burden between 1990 and 2010 and indicated the increased contribution of the nutrition-related risk factors to this burden (Lim et al., 2012). NCDs are closely linked to lifestyle factors including poor dietary patterns, low physical activity, tobacco use and harmful use of alcohol (Kvaavik, Batty, Ursin, Huxley, & Gale, 2010; World Health Organisation, 2010). Therefore, NCDs can be defined as lifestyle-related diseases, which are a consequence of population adaptation to an unnatural environment (Sagner et al., 2014).

The rapid rise in the prevalence of obesity and diet-related NCDs has become a public health concern necessitating attention in both developing and developed countries (Lobstein & Brinsden, 2014). Being overweight or obese is well defined as an NCD-associated chronic health condition that potentially increases the risk of developing coronary heart disease, type 2 diabetes and some cancers. Therefore, new approaches towards disease prevention with emphasis on early interventions need to be widely investigated. A rational approach through nutritional interventions alongside reducing environmental chemical exposures in pre-pregnancy, during pregnancy and also the first few years of life could be the key towards clearer answers on the origins of NCDs (Barouki et al., 2012). The potential that NCDs can be prevented is of significant importance and warrants further investigation. This approach could, in principle, have a very large impact on reducing disease incidence and also the cost of healthcare.

The epidemiology, risk factors and prenatal nutritional preventative approaches to allergies as NCD and obesity as a potential risk factor for NCDs are discussed further in the following sections. The epidemiological data are drawn from childhood studies as this is the area of interest in this programme of research.
2.3. Definition of allergy, symptoms and diagnosis

Clemens von Pirquet, the Viennese paediatrician first coined the term allergy in 1906 originating from the two Greek words “allos” (other or different) and “ergia” (energy or action) (Igea, 2013).

In the current nomenclature for allergy, developed by the appointed Task Force in the European Academy of Allergology and Clinical Immunology (EAACI) (Johansson et al., 2004), hypersensitivity is used as an umbrella term for describing objectively reproducible adverse reaction(s) to a defined stimulus at a dose that is tolerated by normal people. Hypersensitivity is subdivided into allergic and non-allergic hypersensitivity. The main differentiating factor between the two is that allergic hypersensitivity involves adaptive immune system. Based on the mechanism involved, allergic hypersensitivity is further subdivided into those mediated by immunoglobulin E (IgE-mediated) and those not mediated by immunoglobulin E (non IgE-mediated). Inflammation in the latter could be caused by either allergen-specific lymphocytes or IgG immunoglobulin isotype. This is shown in Figure 2.1.

The molecular pathways involved in IgE-mediated allergies are complex and not very well understood. In brief, T cells are key on initiating allergic reactions in the body where there is an imbalance from T regulatory (Treg) cells, also called Th1 cells, towards allergen specific type 2 helper T (Th2) phenotype (Palomares, Akdis, Martin-Fontecha, Akdi, 2017). In other words, while the dominance of Treg cells in healthy individuals inhibits allergic responses to different allergens, a reduced immune suppression could lead to a shift towards Th2 subsets and thus, developing allergies (Akdi, et al., 2004; Palomares, et al., 2014).

Another common term in the field of allergy is “atopy” which describes a genetic predisposition to producing immunoglobulin E in response to allergens, due to personal or familial tendency (Johansson et al., 2001). Likewise, the “atopic march” denotes the natural course of allergic disease in which early onset of atopic dermatitis and food allergy in infancy precedes the developing of wheeze/asthma and allergic rhinitis in childhood (Bantz, Zhou, Zheng, 2014).
Known allergic diseases include asthma, rhinitis, conjunctivitis, atopic dermatitis (eczema), urticaria, food hypersensitivity, drug hypersensitivity, insect sting or bite hypersensitivity and anaphylaxis (Johansson 2004). It should be noted that these are umbrella terms since these diseases could also be initiated by non-IgE-mediated process. Therefore the term allergic disease should be applied when an immunologically mediated sequence is triggered. Asthma is one example of a heterogeneous disease caused by many environmental factors. A genetic predisposition has, however, been established as the main risk factor for developing IgE-mediated asthma to common aeroallergens (Holgate et al., 2013) and this needs to be differentiated as “allergic asthma”. Dermatitis is also an umbrella term for a local inflammation of the skin and when an IgE-immunoglobulin-associated reaction is involved the appropriate term is “atopic eczema” or an “allergic dermatitis”. Similarly, the term “food allergy” should also be applied if immunologic mechanisms are found to be involved.

Allergic diseases can affect individuals at any age, although allergies frequently begin early on in infancy. During the first months of life, infants are prone to developing atopic dermatitis, food allergies and recurrent wheezing, whereas asthma and allergic
rhinitis usually occur later in childhood (Bieber, Leung, Gamal, & Ivancevich, 2013). Allergies can also be triggered by a wide range of indoor and outdoor factors such as foods, pets, mould, insect sting, drug, and pests. Generally speaking, allergens are members of a rather limited number of protein families, which are capable of binding to specific antibodies (van Ree, 2014). Manifestation of allergies to cow’s milk protein (CMP) is the most common in the first few months when the infant is exposed to cow’s milk via breast milk, infant formula or solid foods. Moreover, allergic reactions to foods, particularly in children, are primarily caused by eight foods, namely cow’s milk, egg, wheat, soy, peanut, tree nuts, fish and shellfish (Allen & Koplin, 2012).

A summarised checklist of related physical symptoms of allergic diseases, either single, systemic or in combination, is presented in Table 2.1.
### Table 2.1. Common allergic diseases and their related physical symptoms

<table>
<thead>
<tr>
<th>Asthma</th>
<th>Atopic dermatitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Recurrent episodes of wheezing</td>
<td>- Pruritus</td>
</tr>
<tr>
<td>- Breathlessness</td>
<td>- Facial and patch eczema lesions</td>
</tr>
<tr>
<td>- Chest tightness and cough, particularly at night or the early morning</td>
<td>- Generalised body eczema</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Food allergy</th>
<th>Allergic rhinitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Cutaneous reactions (urticaria, angioedema, contact rash)</td>
<td>- Stuffed-up nose</td>
</tr>
<tr>
<td>- Gastrointestinal reactions (oral allergy syndrome, nausea/vomiting, abdominal pain, diarrhea, constipation, enteritis &amp; colitis)</td>
<td>- Runny nose</td>
</tr>
<tr>
<td>- Respiratory reactions (asthma, rhinoconjunctivitis, laryngeal edema)</td>
<td>- Postnasal drip</td>
</tr>
<tr>
<td>- Other reactions (anaphylaxis)</td>
<td>- Red itching eyes</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Anaphylaxis</th>
<th>Allergic conjunctivitis (ocular allergy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Cutaneous symptoms (itching, flushing, hives, angioedema)</td>
<td>- Itching</td>
</tr>
<tr>
<td>- Respiratory symptoms (shortness of breath, wheeze, cough, rhinitis)</td>
<td>- Tearing</td>
</tr>
<tr>
<td>- Cardiovascular symptoms (dizziness, syncope, hypotension)</td>
<td>- Redness</td>
</tr>
<tr>
<td>- Abdominal symptoms (nausea, vomiting, diarrhoea, cramping pain)</td>
<td>- Chemosis</td>
</tr>
<tr>
<td>- Other symptoms (headache, sub-ternal pain, seizure)</td>
<td>- Lid oedema</td>
</tr>
<tr>
<td></td>
<td>- Photophobia</td>
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</tbody>
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<table>
<thead>
<tr>
<th>Drug hypersensitivity</th>
<th>Urticaria (chronic spontaneous type)</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Skin (urticaria, angioedema)</td>
<td>- Wheals</td>
</tr>
<tr>
<td>- Respiratory (asthma, acute lung infiltrates, hypersensitivity pneumonitis)</td>
<td>- Angioedema</td>
</tr>
<tr>
<td>- Hematologic (eosinophilia, cytopenias)</td>
<td>- Both of the above</td>
</tr>
<tr>
<td>- Hepatic (cholestatic, hepatocellular damage)</td>
<td></td>
</tr>
<tr>
<td>- Renal (glomerulonephritis, nephrotic syndrome)</td>
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<tr>
<td>- Anaphylaxis</td>
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<tr>
<td>- Serum sickness</td>
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<tr>
<td>- Drug fever</td>
<td></td>
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<tr>
<td>- Vasculitis</td>
<td></td>
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</tbody>
</table>

| Insect sting/bite hypersensitivity | |
|-----------------------------------| |
| - Allergic systemic reactions (cutaneous, respiratory, cardiovascular, gastrointestinal) | |
| | - Systemic toxic reactions |

*Adapted from WAO White book and EAACI Atlas*

Management of allergic diseases principally relies on applying appropriate diagnostic methods and identifying the causative allergens. The first step in diagnosis is taking a thorough allergy-focused history, accompanied by physical examination, which should include questions regarding (Sánchez-Borges, Ivancevich, Pérez, & Ansotegui, 2013):

- Present illness and symptoms
- Sources of exposure to allergens or irritants at work or other environment
- Personal or passive tobacco exposure, including a review of previous evaluations and treatments
- The impact of illness
- Review of systems
- Presence of other organ-related diseases and medications
- Psychosocial setting and past medical history
- Prior drug or food allergies as well as the family history

When an adequate medical history suggests sensitisation to causative allergic factors, the next step is for relevant allergen-specific tests to be performed by qualified healthcare professionals trained in allergy. A number of in vivo and in vitro tests are introduced for the diagnosis of allergic diseases. These include immediate-type skin tests, in vitro allergen-specific IgE, basophil-based tests, organ challenge tests, patch tests, total serum IgE, serum tryptase, eosinophil cationic protein, eosinophils in blood and other biologic fluids, additional procedures (including spirometry, bronchoscopy, nitric oxide in exhaled air) and environmental determinations. The Double-Blind Placebo-Controlled Food Challenge (DBPCFC) is the gold standard for the diagnosis of any food allergy (Sampson et al., 2012).

Skin tests are the main in vivo diagnostic tools for IgE-mediated allergies and are easy to perform, reproducible and cheap (Sánchez-Borges, et al., 2013). The tests are either percutaneous or intracutaneous on the skin of the inner forearm or the upper back. If wheal and flare reactions form 15-20 minutes after the introduction of a suspected allergen, an allergic response is developed. The level of response largely depends on the degree of sensitivity, the number of mast cells and the potency of the allergic extract. To minimise the negative and false positive results, positive control (histamine dihydrochloride or phosphate) and a negative control (glycerosaline diluent) is used. A positive test result does not prove clinical allergy on its own and needs to be supported with findings from the medical history and physical examination. In contrast, a negative test result does not necessarily indicate that the disorder is not caused by the suspected allergen. Patients are advised to avoid taking antihistamines and certain medications including topical corticosteroids and tricyclic antidepressants before taking the test to prevent false negative results.
Skin Prick Tests (SPTs), also termed percutaneous or puncture tests, are the most common technique for detecting allergies. A positive result is considered when a wheal response of at least 3mm diameter larger than negative control alone occurs (Sánchez-Borges, et al., 2013). SPTs are identified as the best method for detecting sensitisation to inhalant and food allergens (Genser & Schmid-Grendelmeier, 2014).

Intradermal or intracutaneous tests are 10,000 times more sensitive than SPTs but have higher rates of false positive results. They are typically performed when prick tests are negative in the presence of a clear history of exposure coupled with symptoms and are best known for diagnosis of venom and drug allergies (Genser & Schmid-Grendelmeier, 2014).

*In vitro* tests are the benchmark methods in the diagnosis of allergies (Sánchez-Borges, et al., 2013). The most common of these is a serological test measuring levels of allergen-specific IgE immunoglobulin (sIgE). Raised level of sIgE in the serum indicates that an allergy may exist and such a result needs to be correlated with symptoms and a positive medical history of exposure to allergen.

**2.4. Allergic disorders in children**

As explained, allergy is an immunologically mediated and allergen-specific hypersensitivity manifesting mainly in the skin and mucous membranes. This involves different pathomechanisms and hence different approaches to diagnosis, therapy and prevention can be taken (Ring, 2014).

Allergic diseases have seen a rise worldwide with children suffering the highest burden of the diseases in the last two decades (Kaliner & Giacco, 2013). A time trend analysis for allergic diseases in the UK, not including asthma, showed that they are still amongst the most common disorders (Gupta, Sheikh, Strachan, & Anderson, 2007), accounting for 6% of all GP consultations (the Royal College of Physicians Working Party, 2003). It is estimated that the direct cost of allergic diseases to the NHS is in the order of £1 billion per annum (Gupta, 2004) not including the financial impact of school or workdays lost, lower productivity or diminished quality of life (Department of Health Allergy Services Review Team, 2006).
The most common allergic disorders in children are food allergies, asthma and eczema (Pawankar, Canonica, Holgate, Lockey, & Blaiss, 2013).

*Food allergies*

A systematic review on diagnosing and managing common food allergies concluded that there are difficulties making precise estimates of food allergies due to differing definitions, methods of diagnosis, dietary exposures and geographical variations (Chafen et al., 2010). Nationally representative population-based studies from the US and Canada showed the self-reported prevalence of food allergy in children to be 6.53% in 2007-2010 (McGowan & Keet, 2013) and 6.7% in 2008-2009 (Soller et al., 2012), respectively. Data from an unselected birth cohort of pregnant women on the Isle of Wight, UK also showed that cumulative incidence of food hypersensitivity, defined by food challenges and/or a good clinical history, was estimated 6.8% in children at 10 years old (Venter et al., 2016). A systematic review and meta-analysis of 56 European studies conducted between 2000-2012 found point and overall lifetime prevalence of self-reported food allergies at 5.6% and 17.3%, respectively with higher rate of point prevalence reported in children (Nwaru et al., 2014). The studies included in this review had moderate level of bias due to the heterogeneity in their methodology and diagnostic methods. In general, the major drawback with self-report estimates of food allergy is that they are limited in part by the subjective nature of the data. The changing patterns of food allergy burden in children is investigated in a global collaborative project between the World Allergy Organisation (WAO) and Worldwide Universities Network (WUN) in 2012, targeting the 93 national and regional member societies of WAO (Prescott et al., 2013). Data on food allergy were sourced from the existing data in 89 countries; 12 in Western Europe (including the UK), 5 in Scandinavia and 17 in Central/Eastern Europe, 18 in Asia and Oceania, 15 in the Americas, 10 in the Middle East and 12 in Africa; comprised of 83 WAO member and 6 non-member countries. It should be noted that there was no data on food allergy prevalence in over half of the countries included in the survey, and precise Open Food Challenge (OFC) data was available in only 10% of the countries. Data from this study suggests that some regions in Asia show comparable data for the prevalence of food allergies to European regions and more importantly, there has been an increasing trend of food allergies in both developing and developed countries over
the past 10-15 years. Furthermore, the most common food allergens in children <5 years were reported to be cow’s milk, egg, peanuts and shellfish, with similar patterns in all regions.

Asthma
Asthma is the most prevalent chronic disease in children, with the highest asthma rates reported in the UK and Ireland in both Europe and the world (Gibson, Loddenkemper, Sibille, Lundbäck, & Fletcher, 2013). The International Study of Asthma and Allergies in Childhood (ISAAC) assessed the prevalence and severity of asthma symptoms in its phase three survey in 233 centres from 97 countries using a standardised written questionnaire (Lai et al., 2009). The comprehensive worldwide estimate from this survey in children 6-7 years of age showed the occurrence of severe asthma symptoms ranged from 3.2% in Asia-Pacific and Northern and Eastern Europe to 9.5% in Oceania. A relatively high prevalence (≥7.5%) was also observed in Latin America and English-speaking countries. The most recent study on the global burden of asthma in children (Asher & Pearce, 2014) highlighted prominent differences in asthma symptoms, characterised by wheeze in the past 12 months in children. Some high-prevalence centres in high-income countries showed higher asthma rates from 1990 to the 2000s, and at the same time, higher rates of asthma were also observed in many low and middle-income countries. These numbers indicate an increasing overall burden of asthma worldwide suggesting that there is a universal declining trend for disparities in childhood asthma prevalence.

Eczema
The phase three ISAAC study has also provided updated global data for the prevalence of eczema symptoms in children aged 6-7 years from 143 centres across 60 countries of the 97 countries included (Odhiambo, Williams, Clayton, Robertson, & Asher, 2009). The results indicated that the prevalence of current eczema symptoms ranged from 0.9% in India to 22.5% in Ecuador and that lifetime reported eczema differed between 1.2% in Lithuania and Mexico to 38.6% in Sweden. Higher prevalence of current eczema (≥15%) was reported in five of the world regions including the UK in Western Europe. Data from this survey indicates that eczema (atopic dermatitis) is a major public health concern at a global level.
2.5. Risk factors for developing allergies

Looking at the early origins of allergy, there is evidence that a number of familial, *in utero*, maternal nutritional and environmental factors as well as early postnatal feeding practices are linked to the development of these conditions. In examining this area, the specific number of studies mentioned related to risk factors are based on the search results from Pubmed and also, references of conducted systematic reviews and overview of systematic reviews.

2.5.1. Family history and *in utero* environment for childhood allergy

During pregnancy, genetic and a number of environmental factors impact on the risk of developing allergic diseases in the offspring.

*Familial history of allergic disease*

Familial history of allergic diseases is an influential risk factor in developing allergies in a child (Johansson et al., 2004). It is well documented that allergy can be inherited from first-degree relatives with allergies (Böhme, Wickman, Lennart Nordvall, Svartengren, & Wahlgren, 2003; Kjellman & Johansson, 1976) with additional risk in a child with an atopic mother (Ruiz, Kemeny, & Price, 1992). Data from studies on monozygotic twins suggest that the genetic heredity for asthma varies between 0.36-0.75 with higher risk of heredity for peanut allergy at 0.82 (Koppelman, Los, & Postma, 1999; Sicherer et al., 2000). During pregnancy, the genetic heredity of allergy affects the normal function of the immune system with evident markers at birth. Maternal atopy influences the gene expression of regulatory T-cells ($T_{reg}$) and $T_{h2}$ cytokine levels in cord blood (Liu et al., 2011), leading to an impaired balance of $T_{reg}$ to $T_{h2}$ cells and consequently raised level of IgE and increased reaction to allergens early in life (Fu et al., 2013).

*Maternal obesity*

Several observational studies have assessed the association between Body Mass Index (BMI) and Gestational Weight Gain (GWG) during pregnancy and the development of childhood wheeze and asthma. A systematic review and meta-analysis investigated the evidence from 14 observational studies, published between 1996 to 2013 (Forno, Young, Kumar, Simhan, & Celedón, 2014). The included studies defined and
measured BMI and GWG differently and the outcomes were also varied, reported at various age points. The pooled results showed that maternal overweight or obesity in pregnancy and those with high GWG were even more at risk of having children with asthma or wheeze-ever, independently from offspring’s BMI \( (\text{OR}=1.31; \quad 95\% \text{ CI}=1.16-1.49, \ 11 \text{ studies} \) and \( \text{OR}=1.16; \quad 95\% \text{ CI}=1.00-1.34, \ 5 \text{ studies} \), respectively). High statistical heterogeneity were found in meta-analyses and additionally, studies did not control for the role of potential confounders such as maternal asthma. Further data from large birth cohort studies have shown that maternal pre-pregnancy obesity and high maternal BMI in early pregnancy were associated with an increased risk of developing asthma/wheezing \( (\text{Harskamp-Van Ginkel, London, Magnus, Gademan,} \ 2015) \) and asthma only in the offspring \( (\text{Ekström et al.,} \ 2015) \); however the offspring’s BMI has a mediating role in this association. In general, the evidence from observational studies is limited due to a number of confounders that could affect the associations between maternal obesity and GWG in pregnancy and development of asthma/wheezing in offspring. In fact, the effect of maternal obesity on childhood asthma/wheezing could be explained by a number of direct and indirect mechanisms defined as either causal \( (\text{e.g. atopic family history}) \) or confounder \( (\text{e.g. child’s BMI}) \) factors and need to be considered in prevention strategies.

**Prenatal maternal smoking**

Epigenetic studies have documented that *in utero* tobacco exposure alters the expression of genes that are involved in the onset of childhood asthma \( (\text{Patil et al.,} \ 2013; \text{Scholtens et al.,} \ 2014) \). The effect of exposure to tobacco smoke during pregnancy and developing childhood wheeze and asthma has been studied in a number of observational studies. Data from two cohorts on the influence of grandmaternal smoking during pregnancy with the mother and the risk of developing wheeze/asthma in their grandchildren have shown contradictory findings, with one study showing no effect \( (\text{Miller, Henderson, Northstone, Pembrey,} \ 2014) \), and another study indicting a positive association \( (\text{Magnus et al.,} \ 2015) \). There are currently 79 prospective studies (cohorts and cross-sectional) that investigated the exposure to passive smoke during pregnancy as a risk factor for developing childhood respiratory diseases such as wheeze and asthma. The publication dates of studies ranges from as early as 1985 to 2011 and reported the
defined outcome(s) either as current or lifetime for various age ranges. The studies were conducted both on at risk populations (parents with a history of asthma and/or allergies, low birth infants) and unselected populations. The majority of studies have relied on self-reported data to measure the exposure to passive tobacco smoke, and a few studies provided objective measures i.e. plasma cotinine (Murray et al., 2004). The source of exposure stated as either maternal, paternal/household or both at different times of prenatal, postnatal or both across studies. When estimating the effect of association between smoking and the development of respiratory outcomes, some studies controlled for the role of potential confounders such as dose-response, birth-weight and gestational week in children by conducting adjusted analysis whereas many have relied on unadjusted associations. The largest study is the Danish National Birth Cohort, involving 34,793 mother-child pairs, which reported that smoking during pregnancy, for “less than everyday” and “everyday” independently, predicted the risk of “wheeze ever” in children by 18 months in adjusted analysis (OR=1.22, 95% CI=1.01-1.49) and (OR=1.46, 95% CI=1.36-1.56) respectively (Linneberg, Simonsen, Petersen, Stensballe, & Benn, 2006).

Given the large number of conducted studies and variability in their design and reported outcomes, it is difficult to reach a conclusive result for the association between smoke exposure and development of childhood wheeze and asthma based on individual primary studies. A number of systematic reviews have been conducted on the topic. The most recent review (Silvestri, Franchi, Pistorio, Petecchia, & Rusconi 2015) included a total of 43 papers from 29 birth cohorts that were only carried out on unselected populations and presented the results in two age groups: infants and pre-school children, schoolchildren and adolescents. The reviewers rated methodological quality of some of the included studies as poor, mainly because they did not use adjusted outcome measures. The meta-analysis of 13 studies showed that prenatal exposure to maternal smoking increased the risk of wheezing/asthma in children under 6 years old (OR=1.36; 95% CI=1.19-1.55). When only high-quality studies were included in the meta-analysis, the results remained significant. The pooled estimates were similar for the age of older children over 6 years for wheezing/asthma (OR=1.22, 95% CI=1.03-1.44). The findings from this systematic review are in agreement with the previous review on the topic by Burke, et al., (2012) that included studies on both at risk and unselected populations. Altogether, the studies included in this review were of a different quality and with inconsistent findings for the reported...
outcomes; nevertheless, the results of this review provide evidence-based results of the current literature that prenatal smoking exposure has an influential effect on the risk of developing childhood respiratory diseases.

**Prenatal maternal stress (PNMS)**

Prenatal psychological stress might lead to an increased risk of childhood wheeze and asthma via genome-wide alterations in DNA-methylation (Trump et al., 2016). The role of PNMS on developing allergic conditions in children has been the interest of researchers worldwide. Sixteen studies have addressed the issue so far, published between 2004 and 2014. With the exception of two cross-sectional studies, the remainder were prospective cohort studies. PNMS was measured differently across studies including negative life events, anxiety/depression, bereavement, distress and job strain. Furthermore, PNMS was mostly measured subjectively using self-reported data in the studies and also, at different time-points in pregnancy from first to third trimester. All studies controlled for confounders by conducting adjusted analyses; however, there was a wide heterogeneity between studies for the type of covariates included. The largest population-based cohort from Sweden included 3.2 million births between 1973 and 2004, and assessed the relationship between maternal exposure to death of a spouse or child, either close to pregnancy time or during pregnancy, and risk of hospitalisation for asthma in offspring that were followed-up by December 2006 (Khashan et al., 2012). Its findings indicated that prenatal exposure to bereavement in any exposure period was related to a higher risk of asthma in offspring (RR=1.20; 95% CI=1.03-1.39) and there was a higher risk where bereavement happened during pregnancy or was related to death of a spouse.

In order to provide a more precise understanding for the association between PNMS and atopic diseases in children, a systematic review has been conducted (Andersson et al., 2016). This review included 16 observational human studies and did not perform meta-analysis due to high-heterogeneity observed between studies in terms of the study design, exposures and outcomes. Overall, the authors reported that a positive association between PNMS and different atopic conditions was reported in 21 of the 25 statistical analyses conducted in the included studies. The results of this systematic review suggests a role for adverse life events and stress during pregnancy in the development of allergic conditions in children, which could be explained by the immunomodulatory effects of PNMS on the foetal immune system. Future studies might also consider objective methods for measuring PNMS such as interview-based
approaches, since stress is a complex concept and interviews could provide more reliable information on the nature of stress including the period and severity that it has been experienced.

*Maternal exposure to medicines*

Shaheen and colleagues investigated the potential interactions between prenatal intake of acetaminophen, also known as paracetamol, and maternal antioxidant genotypes on childhood asthma (Shaheen, Newson, Smith, & Henderson, 2010). They reported that the effect could be modified by maternal genotype at antioxidant-encoding genes proposing a causal relationship. Several pathophysiological mechanisms are also described for the association between paracetamol use and childhood asthma such as the glutathione pathway, imbalance of $T_\text{h}1$ to $T_\text{h}2$ ratio and anti-genetic effect mediated by IgE (Lourido-Cebreiro, Salgado, Valdes, & Gonzalez-Barcala, 2017). The use of medicines during pregnancy and their effect on childhood allergies has been scrutinised in a number of population-based studies. To date, there are 10 observational studies that reported prenatal maternal use of paracetamol, as a common pain-reliever and anti-fever drug and its long-lasting effect on the development of asthma in children. The studies were conducted worldwide, published between 2002 to 2013 involving mother-child pairs. There was a wide variation between studies with regard to design (cross-sectional vs. cohort), measuring the time of exposure to paracetamol in pregnancy (early, mid and late stages), frequency of use, controlling for different confounders and different follow-up time-points. The largest study from the Danish National Birth cohort initially involved 90,549 mothers and 12,733 completed the final follow-up questionnaire when their child was 7 years old (Rebordosa, Kogevinas, Sørensen, & Olsen, 2008). Physician-diagnosed asthma at 18 months and seven years was associated with paracetamol use at any time during pregnancy (RR=1.18; 95% CI=1.13-1.23) and (RR=1.15; 95% CI=1.02-1.29) respectively. Taking paracetamol only during the first trimester of pregnancy also elevated the risk of persistent wheezing at 18 months and seven years.

A systematic review and meta-analysis was undertaken to summarise the available evidence on this topic, including only cohort studies that reported asthma outcomes after age 5 years (Cheelo et al., 2015). The review did not include data from cross-sectional studies since these data are at higher risk of recall bias and could dominate the overall findings. Overall, the 11 included studies were rated as adequate for assessment of exposure while the assessment of outcome was not satisfactory in most
studies due to unadjusted analysis conducted. The meta-analysis of five studies showed that any use of paracetamol during the first trimester of pregnancy elevated the risk of childhood asthma (OR=1.39; 95% CI=1.01-1.91); however heterogeneity of 64.2% was observed between studies. A stronger relationship was found for paracetamol use during the second and third trimesters and studies were homogenous (OR=1.49; 95% CI=1.37-1.63, three studies). Overall the heterogeneity observed between studies was because those that conducted adjusted analysis included diverse confounders that might have attenuated the associations. To conclude, the current evidence for the association between maternal use of paracetamol during pregnancy and developing asthma is not definitive since several primary and secondary confounders could affect this association such as recall bias, smoke exposure and respiratory tract infections.

Studies have also looked at the impact of acid-suppressive drugs during pregnancy, Proton Pump Inhibitors (PPIs) and Histamine 2-Receptor Antagonists (H₂RAs), on development of childhood asthma and other allergic diseases in the offspring. Whilst data from some birth cohorts reported an increased risk in unadjusted analysis for asthma and other allergic diseases in the offspring (Andersen et al., 2012; Källén, Finnström, Nygren, & Otterblad Olausson, 2013; Mulder et al., 2014) a very recent study from the Health Improvement Network in the UK on pregnancies between January 1996 to December 2010 showed no significant association for PPIs but only for H₂RAs, after adjusting for the underlying covariates (Cea Soriano, Hernández-Díaz, Johansson, Nagy, & García-Rodríguez, 2016).

Furthermore, a birth cohort from Italy evaluated the role of prenatal exposure to antibiotics and development of wheezing in the offspring and reported only third trimester exposure was associated with an increased risk of “ever and recurrent wheezing” and the effect was attenuated in adjusted analysis (Popovic et al., 2016). Altogether, these findings emphasis the fact that when interpreting the results of in utero exposure to medicines on later childhood allergic diseases, the intrinsic limitations such as differences between study design/power as well as the role of diverse confounders including maternal comorbidities, genetic and environmental factors due to nature of observational studies need to be considered. The current evidence is therefore not definitive and cannot warrant changing the guidelines.
2.5.2. Maternal diet during pregnancy and development of childhood allergies

Data on the effect of prenatal diet and development of allergic diseases in offspring primarily originated from observational studies and thus could largely be biased by a range of confounding factors. Nevertheless, these findings highlight the role of epigenetic mechanisms through which maternal diet during pregnancy may influence foetal gene expression and the development of immune system and metabolic function in children.

Restricted maternal diet during pregnancy could predict the development of allergic diseases later in life. Data from a cohort of pregnant women exposed to famine in the Netherlands during World War II showed that under-nutrition in early and mid gestation increased the risk of obstructive airway disease but not IgE concentrations or lung function in children (Lopuhaä et al., 2000). This finding is further supported by animal studies showing that protein restriction in rats had an adverse effect on respiratory development in their pups (Pike et al., 2014).

Numerous observational studies have assessed the possible association between prenatal nutrition during pregnancy and childhood allergic conditions. There are currently 65 papers published from 1990 to 2014, involving a range of sample sizes and various designs: cross-sectional, case-control and cohort studies. There are wide variations between studies in terms of the type and definition of diet since many have only measured intake of individual food items/micronutrients or consumption of specific food group(s), and others assessed dietary patterns. In addition, only a few studies have conducted adjusted analyses, considering the role of confounders. Moreover, many studies did not systemically record diet during pregnancy, using daily register or Food Frequency Questionnaire(s) (FFQ), and some only collected and/or reported data on individual nutrient intakes. In addition, only a handful of studies have comprehensively assessed maternal dietary intake accompanied by analysing relevant biological markers, either in mother or infant. Likewise, the definition/description of outcomes are largely varied across studies and they report various follow-up durations. Finally, the associations between consumption of different foods or food groups have been inconsistent and contradictory across
studies, possibly because of inconsistencies between the defined foods or food groups. The largest prospective birth cohort is from Denmark, including 60,466 mother-child pairs and collected data on Vitamins A, E and K, using validated FFQ in mid pregnancy. This study showed that “ever admitted asthma” and “current asthma” were related with maternal total Vitamin K intake (highest quintile (Q) vs. lowest: 1.23; 95% CI=1.01-1.50) and (Q5 vs. Q1: 1.30; 95% CI=0.99-1.70) respectively (Maslova, Hansen, Strom, Halldorsson, & Olsen, 2014). Consumption of Vitamin A and E during pregnancy in this study showed a weak inverse association with childhood allergic rhinitis. Studies on maternal dietary patterns during pregnancy have also defined different terminology and reported diverse findings. The largest study was the Avon longitudinal study, in the UK that investigated the association between overall dietary patterns in pregnancy using FFQ completed at 32-weeks of pregnancy and allergic outcomes in offspring (Shaheen, et al., 2009). The study involved 14,062 mother-child pairs and results did not show any associations between dietary patterns and allergic outcomes, adjusted for relevant confounders. To reach a conclusive result, meta-analyses have been conducted to systematically evaluate the current body of evidence for probable associations between maternal diet during pregnancy and development of allergies in offspring. The most recent systematic review included only cohort studies (a total of 32 birth cohorts: 29 prospective and 3 retrospective) that objectively assessed maternal nutrition during pregnancy using systematic recording and reporting development of childhood asthma, wheezing, eczema and other atopic conditions (Beckhaus et al., 2015). The overall quality of included studies was judged as good and satisfactory; however most studies were rated as inadequate for the outcome assessment and follow-up. The review evaluated the associations between consumption of a range of micronutrients (vitamins, magnesium, zinc, selenium, etc.), fruits, vegetables, fish, meat, dairy, fats, Mediterranean diet and other dietary patterns as well as sweetened beverages during pregnancy across cohorts and development of childhood allergies. Pooled estimates of meta-analyses indicated that prenatal intake of Vitamin D (OR=0.58, 95% CI=0.38-0.88, four studies); Vitamin E (OR=0.6; 95% CI=0.46-0.78, five studies) and Zinc (OR=0.62; 95% CI=0.40=0.97, three studies) during pregnancy were protective against development of childhood wheeze. The results of this review did not find any associations between consumption of these or other foods/nutrients/dietary patterns and development of other childhood allergies. Another systematic review that included 42 studies with different designs
(12 cohorts, four case-control and 26 cross-sectional studies) only investigated the
evidence for intake of fruit and vegetables during pregnancy and risk of wheezing and
asthma in children (Seyedrezazadeh et al., 2014). The sub-group meta-analyses for
the study design and fruit/vegetable categories indicated that highest intake of fruits
and vegetables during pregnancy, in either study designs, were protective against
childhood wheeze but not asthma. Collectively, the current evidence for the role of
maternal diet during pregnancy and the development of allergic disorders in offspring
is limited due to high heterogeneity between studies and few studies that could
contribute in meta-analyses. This stresses the need for further robust adequately
powered epidemiological studies and ideally, standard definition of food groups as
well as nutritional patterns. In addition, diet needs to be assessed using validated
dietary methods and preferably be linked to the dietary assessments with biological
analysis. More importantly, there is a paucity of evidence from RCTs that could
potentially address the role of maternal nutritional factors during pregnancy for
prevention of childhood allergies.

2.5.3. Breast-feeding practices and development of childhood allergies
The role of breast-feeding and the prevention of allergic diseases has been assessed in
several observational studies and to date the evidence is not conclusive. This reflects
the discrepancies between the studies with regard to their methodological quality and
design as well as the role of confounding factors such as duration of breast-feeding,
weaning practices, cultural practices in different populations and definition of allergic
outcomes. Another key issue in studies of this type is the need to rely on
observational data alone, as the exposure to breast-feeding cannot be randomised.

To date, there are 89 observational studies that have assessed whether breast-feeding
could influence developing asthma and allergic outcomes in children. The studies are
of different designs, mainly cohorts as well as cross-sectional and case-control,
published between 1981 to 2016. The samples in studies were of small to large scale
and of either at risk or unselected population. There are wide variations between
studies with regard to measurement of exposure to breast-feeding and its duration,
deinition of outcomes and also, duration of follow-up. In addition, substantial
heterogeneity exists between studies for controlling the key confounders such as
socio-economic status and family history of allergic diseases. The largest study is
conducted on two national British birth cohorts and retrospectively investigated the occurrence of wheezing and asthma illness at age 16 in 20,528 children (Lewis et al., 1996). This study did not show a significant association between breast-feeding duration and asthma and/or wheezy bronchitis, in adjusted analyses for risk factors including birth weight and social class. A systematic review and meta-analysis has been conducted to systematically analyse the possible effects of breast-feeding on prevention of childhood allergies, providing an update to the current knowledge in the field (Lodge et al., 2015). This review included 89 observational studies, which comprised of 53 cohort, 33 cross-sectional and 3 case-control studies. The reviewers judged that the evidence from cohort studies were of better quality compared to other study designs and generally, the cohorts and case-control studies were rated as satisfactory to very good quality. Meta-analysis of 29 cohort, cross-sectional and case-control studies showed that duration of breast-feeding, defined as more versus less, was protective against development of childhood asthma in 5-18 years of age (OR=0.90; 95% CI=0.84-0.97); however heterogeneity of 63% was observed between the included studies. Further subgroup analyses of studies by country income revealed some evidence of a reduced risk for asthma at ages 5-18 years with higher duration of breast-feeding in high-income countries although the effect was more prominent in low and middle-income countries (OR=0.86; 95% CI=0.79-0.94). In addition, pooled estimate from five studies stratified by familial history of allergies did not find an association between duration of breast-feeding and developing asthma at 5-18 years. Pooled estimates for other allergic outcomes showed that breast-feeding also decreased the risk of developing allergic rhinitis by ≤5 years of age and eczema by ≤2 years of age; however, high statistical heterogeneity (≥70) was found between studies.

More recent data from the Prediction of Allergies in Taiwanese Children (PATCH) birth cohort reported the effect of breast-feeding on developing childhood allergies (Chiu et al., 2016). The study enrolled 189 children and the follow-up results by 4 years of age showed that exclusive or partial breast-feeding for ≥6 months was associated with lower rate of sensitisation to milk and also eczema in children, only at ages 1 and 1.5 years. The findings from this study also highlights how the sample, exposure and outcome of interest can be defined differently in the conducted studies and therefore, formulating conclusive results is difficult. To summarise, the evidence for the influence of breast-feeding on allergic diseases in offspring is weak and this is
mainly due to high heterogeneity observed between the conducted studies including study design and confounding factors. Measurement of breast-feeding could be at risk of recall bias in cross-sectional studies, and more particularly for allergic outcomes measured later in life such as asthma. Generally speaking, the effect of breast-feeding on allergy is complicated by many factors, including timing of the introduction and type of solid foods (Grimshaw et al., 2013). Population-based studies need to address these confounding factors in order to provide a better understanding for the role of breast-feeding on developing allergic diseases in children.

2.5.4. Introduction of solid foods and development of childhood allergies

Complementary feeding by introduction of solid foods into an infant’s diet, known as weaning in the UK, is a significant landmark in the growth process of children. Throughout this thesis, the terms complementary feeding and weaning will be used interchangeably to describe this process. An overview of the recommendations for infant feeding advice and practices have changed considerably during the last decades as described below:

- 1900: late introduction of solids by 1 year
- 1960s: early introduction of solids <4 months
- 1970s: delayed solids >4 months
- 1990: delayed solids >6 months

It has also been a common practice that the introduction of specific allergenic foods needs to be delayed by up to 3 years. Currently, the Global Strategy for Infant and Young Child Feeding, provided by the World Health Organisation (WHO), recommends that infants need to be exclusively breast fed during the first 6 months of life and commencing nutritive complementary foods afterwards (World Health Organisation, 2003) and continued breast-feeding up to 2 years and beyond if possible (World Health Organisation, 2011). This strategy has been adopted by the UK government, which encourages parents with a more pragmatic approach of around 6 months of exclusive breast-feeding, but the introduction of solids should not be started earlier than 17 weeks of age (Department of Health, 2003).

Whilst current guidelines advise starting solid foods between 4-6 months, with a variety including iron rich foods, there are concerns regarding the delayed timing of high allergenic foods beyond 4 or 6 months. The existing infant feeding information
leaflet for parents in the UK advises that if complementary foods are introduced earlier than 6 months of age, some potentially allergenic foods should be avoided (Department of Health, 2008). During the last decades, cumulative evidence from observational and interventional studies combined with a rising trend of food allergies has raised concerns about the beneficial effect of delayed introduction of highly allergenic foods. As a result, and despite there being no clear guidance for the optimal age of timing of introduction, current guidelines for primary prevention of food allergy do not advise late introduction of allergenic foods (Chan et al., 2014; Fleischer, Spergel, Assa, & Pongracic, 2013; Muraro et al., 2014).

The influence of timing of allergenic food introduction to the infant’s diet on developing allergies in children has been examined in numerous observational and RCTs. There have been around 69 observational studies with different designs of 55 cohort, 2 nested case control studies, 12 case-control or cross-sectional studies, published between 1979 to 2015. Studies were conducted at high risk (atopic) or unselected (non-atopic) population. Exposure was assessed to introduction of a single or multiple high allergenic foods such as cow’s milk, egg, fish and reported various outcome measures for varied age ranges. There were high heterogeneities between studies for defining and assessment of outcome as well as adjustment for potential confounders. The largest cohort study from Israel investigated early exposure to cow’s milk protein in 13,019 infants by telephone interviews and assessed occurrence of allergic outcomes using SPTs or oral challenge (Katz et al., 2010). The study findings showed that regular consumption of cow’s milk in the first 14 days of life was related to a lower risk of developing cow’s milk allergy. Given the large number of studies and inconsistencies between the reported outcomes, a systematic review and meta-analysis appraised the evidence from observational studies on how the timing of introduction of allergenic foods during infancy may influence the risk of childhood allergic diseases (Ierodiakonou et al., 2016). The reviewers judged the methodological quality of many of the included studies as low for outcome assessment and adjustment for potential confounders. In total, timing of fish introduction between 6-9 months and developing food allergy was reported in three studies. Because of statistical heterogeneity, it was not possible to conduct a meta-analysis; nevertheless, findings of all the three studies reported that early fish introduction was associated with lower risk of allergic sensitisation to any allergen or
food allergens. Pooled estimate of four cohort studies in meta-analyses for fish introduction before 6-12 months showed a lower risk for allergic rhinitis either at ≤4 years (OR=0.59; 95% CI=0.40-0.87) or 5-14 years (OR=0.68; 95% CI=0.47-0.98). In addition, meta-analysis of three cohort studies showed an association between fish introduction between 8-12 months and reduced risk of recurrent wheeze at ≤4 years (OR=0.72; 95% CI=0.59-0.87). Despite the fact that the meta-analyses indicate there is an association between fish introduction early in life and development of allergies in children, even pooled, these studies are small and therefore the evidence is not robust. Timing of introduction of other allergenic foods was not associated with developing childhood food allergy, allergic sensitisation or rhinitis and wheeze. The reviewers also reported that timing of allergenic food introduction and risk of eczema was not consistent across the observational studies and did not conduct meta-analyses. To summarise, the current evidence from observational studies for timing of allergenic food introduction and development of food allergies is not certain mainly because of high heterogeneity between studies and the small number of studies contributing to meta-analyses.

There are 24 RCTs that evaluated the efficacy of early introduction of highly allergenic foods in infants and prevention of allergic diseases. The studies were published between 1966 to 2016 and conducted on high-risk or unselected population. These RCTs either introduced multiple or single allergenic foods and the duration of intervention varied from early short-term to early sustained introduction to trials of delayed allergenic food. Some interventions were also multifaceted where dietary components were administered together with environmental control measures such as house dust mite avoidance and tobacco smoke. Most studies compared cow’s milk versus soya milk formula and additionally, many intervention studies for egg introduction and developing egg allergy only published abstracts and did not share full trial findings. Furthermore, the outcome measures were defined and measured differently across studies and reported at various age points. The largest open-label controlled trial was conducted in the US and allocated at birth 1,750 Caucasian children, with a positive or negative family history of allergic diseases, to breast-feed versus either cow’s milk or soya milk groups for six months (Halpern et al., 1973). Half of the babies, regardless of their family history of allergies, were also allocated to take egg yolk before 3 weeks with the other half after 6 months. A physician
assessed the outcome when the children were 7 years old and the incidence of allergy was similar between breast-fed infants with the two other substitute formula groups. The development of allergies was also comparable in the egg yolk groups. Also, Learning Early About Peanut Allergy (LEAP) was the first open-label RCT to assess the efficacy of early introduction of dietary peanut in 640 high-risk infants between 4-11 months as a primary prevention strategy (Du Toit et al., 2015). This study showed the risk of developing peanut allergy (assessed by food challenges) at 5 years was lower in the peanut consumption group compared to the avoidance group (13.7% vs. 1.9%), corresponding to 11.8% absolute difference in risk (95% CI=3.4-20.3). The inconsistencies between the conducted RCTs have prompted researchers to conduct a systematic evaluation of the current literature and Ierodiakonou and colleagues (2016) also included RCTs in their review. The reviewers rated the methodological quality of included interventional studies as low in almost 42% of studies because of selection (biased selection of individuals, thus affecting proper randomisation) and attrition (unequal loss of participants) bias as well as assessment of outcome(s). Of note, the study by Halpern et al. (1973) was not included in the meta-analyses since the paper did not report denominators for outcome data. The meta-analysis of evidence on five interventions, with a moderate heterogeneity, indicated that early introduction to egg between 4-6 months compared to later introduction decreased the risk of egg allergy (RR=0.56; 95% CI=0.36-0.87). The sensitivity analysis conducted by excluding studies with only abstract publication yielded comparable results. The pooled estimate from four trials did not show an association between timing of egg introduction and egg sensitisation. Because there were sufficient egg introduction studies, the reviewers conducted a further heterogeneity-adjusted trial sequential analysis and reported that further large trials are needed to conclude that early egg introduction lowers the risk of childhood egg allergy by at least 30% (relative risk). Moreover, meta-analysis of two RCTS indicated there was a protective effect from introducing peanut between 4 to 11 months compared to later introduction and developing peanut allergy (RR=0.29; 95% CI=0.11=0.74); however high heterogeneity of 66% was found. In general, no associations were found for timing of introduction of cow’s milk or other allergenic foods and, individual allergic food with developing childhood food allergy or allergic sensitisation. In addition, timing of allergenic food introduction was not associated with the risk of allergic rhinitis, wheeze and eczema in the offspring. Collectively, data from this systematic review showed statistically
significant associations only for egg and peanut allergies in offspring. The results of heterogeneity-adjusted trial sequential analysis for egg introduction trials warrant further trials to quantify the scale of treatment effect. Although there were a small number of studies included in meta-analyses and the studied populations and interventions were varied across trials the results of this review provides an evidence-base that suggests current guidelines may need to revise their advice for delayed introduction of some allergenic foods.

Emerging evidence from conducted research is, to some extent, translated into clinical practice. The National Institute of Allergy and Infectious Disease (NIAID) have included an addendum to their guideline recommending high-risk children need to be introduced to peanut-containing foods as early as 4-6 months of age; however, it concluded that the current evidence regarding early introduction of egg in not sufficient (Togias et al., 2017). The recent Scientific Advisory Committee on Nutrition (SACN) report in the UK suggested that exclusive breast-feeding to around 6 months old in children should remain as before, and the introduction of peanut and/or egg needs to start along with other solids (Scientific Advisory Committee on Nutrition, 2017). It appears the weaning debate about early introduction of allergenic foods continues; however the consistent messages are: being developmentally ready is the crucial step to start weaning; introduction of allergenic food need not be delayed; and once introduced, allergenic food should be continued to reduce the risk of food allergies.

2.5.5. Postnatal environmental factors and development of childhood allergies

A number of indoor and outdoor environmental factors in early life are also deemed to be risk factors for developing allergic disorders later in life.

Postnatal smoking

Postnatal exposure to either maternal or parental smoking and risk of developing asthma and wheezing in offspring was investigated in 29 observational studies (cohort and cross-sectional), published between 1989 to 2001. Studies are of small to large scale, conducted on at risk and unselected populations, defined and measured exposure to smoking as well as the outcomes differently. The effect estimates for the
associations between smoking and developing asthma/wheeze were reported either unadjusted or adjusted for varied confounders and reported for various age groups. A systematic review and meta-analysis conducted by Silvestri and colleagues (Silvestri, et al., 2015) assessed the evidence only from 19 cohort studies that reported exposure to postnatal smoking and development of asthma/wheeze in offspring. Pooled estimates of meta-analyses for postnatal exposure to maternal and parental smoking separately showed an increased risk for wheezing in the past 12 months (OR=1.21; 95% CI=1.13-1.31, eight studies) and (OR=1.30; 95% CI=1.13-1.51, seven studies) respectively. Also, eight studies investigated the association between postnatal smoke exposure and asthma or wheezing in schoolchildren and adolescence. There were large inconsistencies between these studies with regards to exposure and outcome and therefore, it was not possible to conduct a meta-analysis. Collectively, these data originate from observational studies that relied on self-reported data and therefore, are exposed to recall bias and misclassification of smoking behaviour that could have underestimated the effects of postnatal smoke exposure. Large statistical heterogeneity was also found between the included studies in meta-analyses. This systematic review only included cohort studies as the strongest observational study design that are less exposed to different types of bias than cross-sectional studies; nevertheless heterogeneity in the included studies such as measures of exposure and outcome did not allow estimating the pooled effect for longer term effects of postnatal smoke exposure and childhood asthma and/or wheeze. This highlights the need for larger cohort studies that could assess the longer-term effects of postnatal exposure and childhood asthma/wheeze.

Living environment (the farm-effect)
Documented evidence suggests maternal farm exposure during pregnancy regulates immunomodulatory responses in the offspring, which is linked to an elevation in the number and function of T_{reg} cells in cord blood resulting in suppression of pro-allergic immune responses i.e. T_{H2} cytokines (Schaub et al., 2009). The role of underlying immunologic mechanisms of postnatal farm exposure on the risk of developing childhood allergies is also assessed in the Protection against Allergy: Study in Rural Environments (PASTURE) birth cohort in a sub-sample of farm-exposed versus non-farm-exposed children at 4.5 years from 5 European countries (Lluis et al., 2014). The study findings verified that farming environment and particularly intake of farm milk
increases the $T_{reg}$ cell numbers and activated T-cell-numbers that is inversely associated with childhood atopic sensitisation and asthma. Over 50 epidemiological studies have examined the associations between growing-up on a farm and childhood and/or adulthood wheeze/asthma and allergic sensitisation. Studies are of small to large scale, published as early as 1999 and are mainly cross-sectional. Across studies, exposure to farm living were measured differently (farm residence, parental occupation or combination) and the definition and measurement of outcome varied. The disease prevalence data reported for different age groups and the effect estimates were controlled for confounders such as age, gender, and familial history in few of the studies, and different associations reported. The largest study was conducted in rural regions of Germany, Austria and Switzerland and is part of the multidisciplinary study to identify the genetic and environmental causes of asthma in these European countries (Loss et al., 2011). The study investigated the effect of farm milk consumption and other farm-related exposures among 8,334 school-aged children and serum samples were provided by 7,606 to assess sIgE levels. Consumption of raw milk showed protective associations, independent from other farm-exposures with developing childhood asthma, atopy and hay fever (adjusted OR=0.59, 0.74 and 0.51 respectively). Intake of boiled farm milk as well as viable bacterial count and total fat content of milk did not show significant associations whereas increased levels of the whey proteins Bovine Serum Albumin (BSA), alpha-lactalbumin and beta-lactoglobulin reduced the risk of developing asthma but not atopy.

A systematic review and meta-analysis was conducted to assess the evidence for the farm-effect from a range of epidemiological studies (Genuneit, 2012). The review included 39 studies published by September 2011. Methodological flaws were reported in the included studies such as sample size and reporting of crude estimates. Meta-analyses of 11 studies showed a protective effect of farm-residence on doctor-diagnosed asthma (OR=0.77; 95% CI=0.60-0.99); however high statistical heterogeneity of 68% was observed between the included studies. Pooled estimates of all studies which defined farm-exposure differently also yielded similar results with higher heterogeneity of 76%. Including only studies with a good methodological quality, conducted in and around the Alps in Europe, decreased the between study heterogeneity for doctor-diagnosed asthma (52%) and current wheeze (0%). Altogether, current evidence confirms the farm-effect as a protective factor for
childhood allergies despite various sources of heterogeneity between the conducted studies. It can be hypothesised that early contact with livestock and consuming unprocessed cow’s milk leads to a much more diverse microbiome in children living on a farm which is a strong protector for asthma and other allergies. Further research could address the sources of heterogeneity across studies more rigorously, assessing the farm-effect on sub-phenotypes of asthma and allergies.

*Other living environmental exposures*

Several epidemiological studies, published as early as 1995, have investigated whether pet ownership could be related to developing childhood asthma and/or allergic sensitisation. Many studies are cross-sectional, conducted among various populations from small to larger scale and defined various classifications of exposure. Diverse findings have been reported from these studies, and few considered the role of confounders; therefore, it is difficult to make a definite conclusion on the association. Here, data are reported from birth cohorts that are exposed to lower bias and could report more reliable data. A US birth cohort study, the Childhood Origins of Asthma (COAST) study, assessed the role of dog ownership in 289 children with a familial history of allergies, by 3 years age (Bufford et al., 2008). The results showed that dog exposure in infancy and particularly around birth reduced the risk of developing AD and wheeze and it was also associated with changes in immune development such as Interleukin-5 (IL-5) and IL-13 responses. The findings from this study are limited because it involved a small sample of children at risk of developing allergies and the findings cannot be generalised to other populations.

To advance the current understanding on this topic, a systematic review and meta-analysis of a large database from 11 European birth cohorts examined the relationship between exposure to pets in early childhood and development of childhood allergies by 6-10 years age (Lødrup Carlsen et al., 2012). The heterogeneities between studies for the definition of exposure and outcome reported for various age groups was minimised in this review since the reviewers were able to collect and combine individual participant data from 11 birth cohorts rather than using published risk estimates. The pooled estimates of the 11 studies did not find an association between any pet ownership (cats, dogs, birds and rodents) during the first 2 years of life and development of asthma, allergic asthma or allergic rhinitis at school age, comparable
results were shown for children with and without allergies. There was some evidence that keeping furry pets could prevent sensitisation to aeroallergens. Collectively, data from this review could have been biased by the method of data collection because some cohorts relied on parent-reported questionnaires for allergic diseases and additionally, number of pets at home was not asked for in most cohorts. Another point is the cultural differences as well as different life-styles could affect keeping some specific types of pets and this could limit findings from different populations. Further analyses combining large birth cohorts from different regions might provide stronger evidence for the association between early pet exposure and developing childhood allergies.

Indoor exposure to mould and dampness in early life as a risk factor in developing childhood allergies has also been studied in over 70 epidemiological studies. Most studies have reported the exposure response relationships, whilst some reported exposure to mold endotoxins. The studies are of various designs (cohorts, case-control, cross-sectional), from small to large scale and conducted on at risk or unselected populations. The definition of outcome and its measurement varied across studies and were reported at different age points. The Pollution and the Young (PATY) study is the largest survey that assessed exposure to visible indoor mold and childhood respiratory health from 12 cross-sectional studies conducted in Russia, North America and 10 Eastern and Western European countries (Antova et al., 2008). Exposure to mold showed a direct association with developing respiratory diseases in children, including nocturnal and morning cough in adjusted analyses and the associations were consistent across studies. Considering the diversity of the reported outcomes in conducted studies, there is no clear direction for the associations between mold exposure and childhood allergies. A systematic review and meta-analysis of studies published “between January 1980 to July 2010” assessed the association between domestic mold and mold components with childhood asthma and allergies (Tischer, Chen, & Heinrich, 2011). The review included 61 studies of different designs that only reported exposure response relationships. Pooled estimate of different study designs showed that domestic visible mold exposure was associated with an increased risk of childhood asthma (OR=1.49; 95% CI=1.28-1.72, 21 studies), wheeze (OR=1.68; 95% CI=1.48-1.90, 19 studies) and allergic rhinitis (OR=1.39; 95% CI=1.28-1.51, 10 studies). The result for exposure to mould-derived components
and allergic diseases in children remains inconclusive because there were a small number of studies and it was not possible to conduct a meta-analysis. To summarise, the findings from this review might be limited by including various study designs and allergic outcomes measured for different age groups; nevertheless the findings indicate a positive association between exposure to mold and development of childhood allergies. Further research might address measuring specific microbial biomarkers at home using longitudinal studies with longer follow-ups to allow for better measurement of longer-term health effects in children.

2.6. Prenatal nutritional approaches for prevention of childhood allergies

The focus of first studies in this PhD research (the systematic reviews) has been on nutritional interventions during pregnancy and the prevention of allergic disorders. The inclusion criteria for interventions were that they solely started during pregnancy and could either terminated at delivery or continued after birth in mothers, infant or both. Therefore postnatal nutritional interventions either in infants or mothers e.g. consuming hydrolised formula, maternal exclusion diets during lactation are beyond the scope of this PhD and not discussed in the following sections.

2.6.1. Dietary restrictions for pregnant women

For decades the primary prevention of allergic disorders in infants was reliant on the concept of reducing or completely avoiding highly allergenic foods such as dairy products and peanuts during pregnancy particularly in women with a family history of atopy. It was believed that prenatal exposure to allergenic foods could have modulating effects on the programming of the immune system in the growing foetus leading to sensitisation (Zeiger, 2003) and therefore an elimination diet during pregnancy could curtail developing allergies early in life. Although there is good evidence that food allergens from maternal diet could cross the placenta, the mechanisms of foetal immune responses to food allergens is not well understood (Edelbauer et al., 2004). Moreover, the current evidence from a systematic review on reduction or avoidance interventions during pregnancy for prevention of allergies has questioned the significance of these strategies (Kramer & Kakuma, 2012). Growing evidence suggests that early exposure to allergens could protect against the
development of allergies in infants (Bunyavanich et al., 2014; Frazier, Camargo, Malspeis, Willett, & Young, 2014; Young, 2015).

For the purpose of this PhD, a systematic review of prenatal interventions on the modification of diet during pregnancy was conducted using a different inclusion criteria to the existing systematic review (Kramer & Kakuma, 2012).

2.6.2. Nutritional interventions during pregnancy
A number of prenatal nutritional approaches have been introduced for prevention of allergies in infants. To avoided bias caused by selecting specific dietary interventions, all interventions were included in the review i.e. probiotics, fatty acids and vitamins.

Foetal origins of allergy indicate that infants with allergy have an altered balance of gut microorganisms early in life (Nauta, Ben Amor, Knol, Garssen, & van der Beek, 2013), and it is thought that this may lead to them developing other allergic outcomes such as asthma (Abrahamsson et al., 2014) and atopy later in life (Fujimura et al., 2016). Since early exposure to the maternal microbiom in infants starts during pregnancy and continues through delivery until after birth, the microbial diversity in mothers plays an important role in the healthy balance of microorganisms and development of immune system in infants (Rautava, Luoto, Salminen, & Isolauri, 2012). This has prompted an interest in whether early interventions involving probiotics in pregnant women might promote developing a healthy immune system in the foetus, with an impact on subsequent risk of allergic diseases in infants (Nylund et al., 2013; Renz, Brandtzaeg, & Hornef, 2012).

The Long-Chain Poly-Unsaturated Fatty acids (PUFAs) family, mostly known by omega-3 (n-3) and omega-6 (n-6) products, are involved in immune-regulation and inflammatory pathways (Tilley, Coffman, & Koller, 2001). The n-3 fatty acids include Eicosapentaenoic Acid (EPA), Docosapentaenoic Acid (DPA) and Docosahexanoic Acid (DHA), and are recognised as having anti-inflammatory properties. The n-6 fatty acids, principally Arachidonic Acid (AA), are identified as having pro-inflammatory effects (Calder, 2011, 2014). Western diet is mainly shifted towards higher intake of n-6 PUFAs by increased consumption of vegetable oils and a corresponding decrease in intake of foods rich in n-3 PUFAs e.g. seafood and oily
fish. Higher concentrations of AA (n-6 PUFAs) promote the production of inflammatory mediators such as prostaglandins and leukotriene, which compete with the synthesis of EPA in cellular phospholipids membranes. Subsequently, a diet rich in sources of EPA and DHA could lead to a reduction in inflammatory mediators and also alter the balance of T_{H1} to T_{H2} (Meydani et al., 1991; Thies et al., 2001; Trebble, Wootton, Miles, & Mullee, 2003), a recognised hallmark of allergic diseases. Furthermore, observational studies summarised in a narrative systematic review (Kremmyda et al., 2011) has shown an association between lower levels of oily fish intake during pregnancy and the development of allergies in children. Collectively, these data suggest that n-3 fatty acid supplementation during pregnancy may hold promise as a strategy to reduce childhood allergies.

Vitamin D plays an important role in the immune system and also the development and function of lung, and a recent review including both animal models and human studies discussed the many roles that Vitamin D could promise for the prevention of allergic diseases (Litonjua, 2009). Animal models have also shown that intake of antioxidants such as vitamin C might protect the growth and development of lung in infants exposed to maternal nicotine (Maritz & Rayise, 2011). Moreover, data from a systematic review on observational studies indicated that consumption of vitamins is associated with a lower risk of allergic diseases (Nwaru et al., 2014). More recent observational studies have also proposed the potential that Vitamin D could prevent allergic respiratory diseases and other allergies (Baiz Nour, Dargent-Molina, Wark, Souberbielle, Annesi-Maesano, & Parkville, 2014; Chawes et al., 2014; Jones, Palmer, Zhang, & Prescott, 2012; Morales et al., 2012). Together, these data form the foundation of the hypothesis that intake of vitamins in pregnant women could protect against developing asthma and other allergic diseases in the offspring.

### 2.6.3. Summary of the literature review for allergic diseases

Allergic diseases are complex conditions and are a product of hereditary tendency along with numerous dietary and environmental exposures including contact with allergens. This multifactorial mechanism for developing allergies suggests that gene expression involved in developing allergies could largely be regulated in response to environmental stimuli, termed epigenetics. Although there is a gap in our current
understanding of the whole range of factors, exposures and interactional effects in allergy risk, appreciating the multifaceted nature of allergies points out the importance of very early preventive interventions as well as early development of therapeutic approaches in at risk infants (Lockett, Huoman, & Holloway, 2015).

### 2.7. Obesity in children

The word “obesity” first appeared in the English language in the 17th century as a descriptive word for excessive fatness (Eknoyan, 2006). Excessive accumulation of fat in the body that could impair an individual’s health is defined as obesity (The World Health Organisation, 2014). The identification of obesity as a condition that could cause pathophysiological complications dates back a century to when its health consequences were first recognised (Beller, 1977; Bray, 1973). In theory, obesity as an actual disease was first recognised by the WHO in 1948, including it in the International Classification of Diseases (ICD) (James, 2008).

The exponential rise in the incidence of obesity over the past few decades has led WHO to declare it as a global public health crisis demanding immediate actions (The World Health Organisation, 2005). Obesity rates have almost doubled since 1980 with an estimated 13% of the world’s adult population being obese in 2014 (The World Health Organisation, 2014). A systematic review of published papers from 1990 to June 2009 estimated that the global direct costs of obesity is between 0.7-2.8% of a country’s total healthcare costs (Withrow & Alter, 2011). In the UK, the overall number of obese people has tripled throughout the last 20 years (National Audit Office, 2001), based on the recent data, the financial burden on the NHS of being overweight or obese is estimated about £5.1 billion (Scarborough et al., 2011). Meanwhile, the indirect costs of obesity to businesses are also important due to poor productivity and absence from work (Butland et al., 2007).

High prevalence of childhood obesity is a serious public health issue and is reported as a potential risk factor for a range of morbidities and medical conditions that occur later in life (European Association for the Study of Obesity, 2015; Kipping, Jago, & Lawlor, 2008). The latest figures of the National Child Measurement Programme
(NCMP) in the UK showed that 19.1% and 14.2% of children aged 10-11 years were obese and overweight respectively (Lifestyle statistical team, 2015). This programme also reports that 9.1% and 12.8% of children at 4-5 years are obese and overweight respectively.

2.8. Obesity complications, diagnosis and the subgroups

Nowadays obesity is well recognised as a chronic disease that can cause a number of health problems but principally diabetes, cardiovascular diseases and several types of cancer (Bray, 2004; Haslam & Philip, 2005). A systematic review and meta-analysis of 207 published cohort studies revealed that obesity and being overweight, with a J-shaped dose-response, are a risk factor for all cause mortality (Aune et al., 2016). In addition, this systematic review showed the lowest dose-response curve was observed among “never smokers” and “healthy never smokers” with BMI ranging between 23-24 and 22-23 respectively. Furthermore it has been shown that regional fat stored in the visceral depots i.e. central obesity, compared to subcutaneous fat, increases the risk of medical disorders (Kissebah & Krakower, 1994; Mauriège et al., 1993). Children with obesity also face developing a number of health and psychological morbidities and are prone to developing several medical conditions in adulthood including type-2 diabetes (Diabetes, 2010; The, Richardson, & Gordon-Larsen, 2013), asthma (Egan & Ettinger, 2013), obstructive sleep apnoea (Narang & Mathew, 2012) and musculoskeletal disorders (Paulis, Silva, Koes, & Van Middelkoop, 2014). It is well documented that about 40% of overweight children could develop overweight/obesity in adolescence and 75-80% of obese adolescents will continue to become obese adults (Lifshitz, 2008).

BMI is the most common anthropometric index for measuring obesity indirectly and is practical at the population level (The World Health Organisation, 2014). The agreed internationally thresholds identify a BMI ≥25 as “overweight” and BMI≥30 as “obese” in adults and is calculated by a person’s body weight (kg) divided by height (metres) squared. The term BMI was first coined by Alphonse Quetelet, a Belgian astronomer and statistician who defined the average man by measuring weights and heights of the French and Scottish armies (Eknoyan, 2006). The appropriateness of BMI for differentiating between different types of obese individuals has been argued.
A recent survey has conducted a cluster analysis on the Yorkshire Health Study, from 2010 to 2012, including the demographic and behavioural information of individuals with BMI≥30 (Green et al., 2016). The analysis in this study found six types of obese people as heavy drinking males, younger healthy females, affluent healthy elderly, the physically sick but contented elderly, unhappy anxious middle-aged and individuals with the poorest health. This observed heterogeneity in individuals with obesity has important policy implications that could allow for more targeted approaches to obesity at a general level.

In children, the classification of BMI needs to be adjusted for age and sex based on the WHO child growth standards (The World Health Organisation, 2014). In the UK, the Scientific Advisory Committee on Nutrition (SACN) recommended that the WHO 2006 child growth standards for infants and children should be used between 2 weeks and 24 months. Then, the 1990 UK reference charts can be applied from age 2 onwards (Scientific Advisory Committee on Nutrition, 2007). A number of methods are used for measurement of childhood obesity. Direct measurements, for example by dual energy X-ray absorptiometry, have a higher accuracy compared to indirect approaches, but are not appropriate for wide spread use at the population level (Goran & Goran, 1998). In this situation, indirect measures such as BMI, skinfolds and waist circumference (Goran & Goran, 1998) including bio-impedance methods such as BodPod and PeaPod are more practical and provide more reliable measures on body composition that are better suited for research surveys (Ma et al., 2004).

2.9. Risk factors for developing obesity

Both genetic and environmental factors during pre-conception and pregnancy as well as early life factors may impact on the risk of developing obesity in a child. In examining this area, the specific number of studies mentioned related to risk factors are based on the search results from Pubmed and also, references of conducted systematic reviews and overview of systematic reviews.

2.9.1. Genetic and in utero environment as a risk factor for childhood obesity

Evidence for a genetic component in obesity has come in part from studies on twins. A systematic review of studies on monozygotic and dizygotic pairs, using structural equation modeling, showed the heritability of BMI ranged from 61-80% over all age
categories in both sexes whereas the distinct environmental influences elevated from 14-40% with increasing age (Nan et al., 2012). Furthermore, observational studies have indicated that parental obesity independently predicts the risk of their children being overweight (Agras, Hammer, McNicholas, & Kraemer, 2004; Fuemmeler, Lovelady, Zucker, & Østbye, 2013). Rankinen et al. (2006) have reviewed candidate genes for human obesity that have been identified through a variety of approaches including Mendelian syndromes, linkage studies and genetic association studies. However the evidence for most of these candidate genes is obtained from studies with a small sample size and a low number of genetic variants tested per gene and hence, consistent associations are reliable for only a handful of the listed genes. The advent of Genome-Wide Association Studies (GWAS) has provided a strong indication for the role of certain genetic variants in the development of obesity and its major complication metabolic syndrome (Aguilera, Olza, & Gil, 2013). Interestingly, data from 10 years of Finnish twin research into obesity showed that despite the high genetic predisposition for weight gain, the risk can largely be modified by environmental factors and maintaining an active lifestyle (Naukkarinen, Rissanen, Kaprio, & Pietiläinen, 2012). A survey from the US also assessed how genetic risk score for high BMI could have differed in response to the obesity epidemic in two birth cohorts born between 1900-1958 and 1992-2014 (Walter et al., 2016). Their findings indicated that the role of the genetic risk score was stronger in the cohort born later, suggesting a greater role for genetic components in an obesogenic environment.

**Maternal obesity**

Well-documented research indicates maternal obesity in pregnancy influences maturation of the neonate’s immune system by reducing the number and function of key innate immune cells in cord blood which in turn correlate with an elevated risk of chronic inflammatory diseases in offspring (Wilson et al., 2015). Epigenetic studies also indicate there are differences in DNA methylation in the offspring of obese versus healthy BMI mothers during pregnancy (Sharp et al., 2015) and this observation is more evident in particular candidate-genes for obesity i.e. Aryl-Hydrocarbon Receptor Repressor (AHRR) (Burris et al., 2015). Growing evidence from epidemiological studies suggests that maternal pre-pregnancy obesity and excessive GWG are closely linked to a higher risk of obesity in children. A review of
the systematic reviews for the early predictors of obesity in children listed high maternal BMI and being overweight prior to pregnancy as potential risk factors for childhood obesity (Cameron et al., 2015). The evidence is based, however, on observational studies that do not imply a causal association and, furthermore, originates from studies with different sample sizes and study design with few being controlled for potential confounders. Furthermore, a systematic review and meta-analysis of 30 cohort and cross-sectional studies showed that maternal obesity is associated with the risk of foetal macrosomia, ≥4000gr weight at birth (Gaudet, Ferraro, Wen, & Walker, 2014). It is worth mentioning that birth weight alone does not reflect either foetal growth trajectory or body composition. A number of studies investigated the associations between GWG and developing childhood obesity. The studies are mainly cohorts that collected data either prospectively or retrospectively, from various socio-economic and ethnic backgrounds and have reported mixed results. The definition and measurement of exposure and outcome are different across studies. A systematic review has endeavoured to provide an evidence from studies that reported GWG as an exposure for childhood obesity (Mamun, Mannan, & Doi, 2014). The review included 15 studies and the pooled estimate of 12 studies showed that excessive GWG is a strong predictor of childhood obesity, from early life to 18 years and above (RR=1.40; 95% CI=1.23-1.59). Furthermore, the risk is higher when the excess weight is gained during early to mid-pregnancy, as reported in another review (Lau, Liu, Archer, McDonald, & Liu, 2014). To conclude, data from epigenetic and epidemiological studies strongly suggest maternal obesity as a risk factor for childhood obesity that initiates epigenetic changes as an underlying mechanism for obesity in the offspring.

*Gestation diabetes mellitus*

Gestational Diabetes Mellitus (GDM) is also identified as a risk factor for childhood obesity. It has been shown that maternal glycaemia has a role in epigenetic mediation of leptin, an adipokine responsible for regulating energy balance, in the foetus (Allard et al., 2015). A synthesis of evidence from observational surveys investigated how maternal diabetes including gestational diabetes could influence the risk of obesity in children (Logan, Gale, Hyde, Santhakumaran, & Modi, 2017). Meta-analysis of three studies showed infants of mothers with GDM had a higher fat mass but lower fat free mass in early days of life. Collectively, data from epigenetic and observational studies
suggest a strong role for GDM as a risk factor for childhood obesity, although the longer-term health risks need to be established.

**Maternal smoking**

Some childhood obesity may also be explained by maternal smoking during pregnancy. There is robust evidence that prenatal exposure to maternal smoking is linked to altered DNA methylation of AHRR in the offspring (Burris et al., 2015; Joubert et al., 2012). The effect of exposure to prenatal smoking on childhood obesity is assessed in numerous observational studies. Studies are of different design, include small to large samples and have been conducted in different world regions. Studies were heterogeneous for measurement of exposure and outcome and had varied duration of follow-up. Only a few studies controlled their analyses for confounders and a range of covariates were considered. The Nurses’ Health Study II is the largest birth cohort in US that assessed the effect of prenatal exposure to parental smoking and obesity in 35,370 children by 18 years of age (Harris, Willett, & Michels, 2013). Adjusted analysis for socio-economic and behavioural variables showed that maternal smoking during pregnancy for doses of 1-14, 15-24 and >25 cigarettes per day increased the risk of obesity by 18 years age (OR=1.26; 95% CI=1.16-1.37), (OR=1.46; 95% CI=1.30-1.63) and (OR=1.43; 95% CI=1.10-1.86) respectively. The influence of prenatal smoking on childhood obesity is systematically investigated in a recent meta-analysis of 39 observational studies (Rayfield & Plugge, 2017). The quality of included studies was rated as good for most studies. The pooled estimate of nine studies showed an association between maternal smoking during pregnancy and development of childhood obesity (adjusted OR=1.55; 95% CI=1.40-1.73). To summarise, the current evidence implies that prenatal maternal smoking is a strong contributor for childhood obesity and suggests that smoking cessation during pregnancy could be encouraged as an effective preventive approach.

**2.9.2. Maternal diet during pregnancy and development of childhood obesity**

Extensive research has explored the influence of maternal intake of various nutrients and dietary patterns during pregnancy specifically on infant’s birth weight and these are discussed in Chapter 5. The focus of this section is on studies that assessed how maternal diet during pregnancy could contribute to childhood obesity in whole life.
This has been the subject of a number of observational studies with an interest in the intake of particular foods. Maternal fat consumption in pregnancy as a risk factor for developing obesity in children is largely examined in birth cohorts. Measurement of exposure and its intake in pregnancy (first, mid or late trimesters) as well as outcome measures and the duration of follow-up varied across studies. A large discrepancy exists between the reported results for obesity outcome in children where some studies have reported beneficial effects and others reported no associations. This could be because of a number of factors such as study power and role of confounders. Therefore, there is a lack of clear evidence for prenatal consumption of fatty acids and childhood obesity. A systematic review and meta-analysis from 15 European and the US birth cohorts was conducted to assess the effect of fish intake during pregnancy, a major dietary source of LC-PUFA, and its influence on childhood BMI trajectories, with 2-year follow-up intervals until the age of 6 years (Stratakis et al., 2016). Of the 19 eligible European birth cohorts, 14 studies included in this review and individual data by 6 years age, with 2-year follow-up intervals was harmonised and combined by reviewers. It was shown that children born to mothers with higher intake of fish prenatally, more than 3 times/week, versus lower fish intake, had higher BMI values from infancy through mid childhood. Also, pooled adjusted results showed fish intake >3 times/week was associated with rapid growth in infants (OR=1.22; 95% CI=1.05-1.42) and elevated risk of overweight/obesity at 4 and 6 years (OR=1.14; 95% CI=0.99-1.32) and (OR=1.22; 95% CI=1.01-1.47) respectively. This finding can be explained by the programming influence of PUFAs during the intrauterine period that could lead to higher growth of adipocytes in the foetus. Despite differences in consumption levels of fish across Europe and the US, the findings of this review provide evidence for the role of high intake of n-3 PUFAs on childhood obesity.

The prospective Finnish birth cohort, not included in the review discussed above, has also examined the effect of dietary fatty acids consumption in late pregnancy on childhood obesity between 2-7 years (Hakola et al., 2016). In girls, a U-shaped association was observed and the lowest and highest quartiles of the n-6/n-3 ratio in maternal diet, compared to the two middle quartiles which were associated with greater adjusted probability of obesity. In boys, the AR/DHA+EPA ratio showed a relation with obesity in the adjusted analysis. Additionally, neither saturated nor mono-unsaturated fatty acids were related to obesity in either sex. This cohort is the
first study that has reported associations between childhood obesity measures and individual fatty acids by means of multiple testing. These results could also reflect the cultural differences in dietary behaviours. For example, the intake of seafood and plant n-3 PUFAs in Finland are among the highest in world whereas intake of n-6 is at the lowest.

The Canadian Healthy Infant Longitudinal Development (CHILD) birth cohort also investigated the relationship between consumption of artificially sweetened beverages during pregnancy and the infant’s BMI at 1-year of age (Azad et al., 2016). An adjusted analysis in this study showed that regular intake versus no-consumption of artificially sweetened beverages in pregnant women increased the risk of being overweight by 2-fold in the offspring (OR=2.19; 95% CI=1.23-3.88). Furthermore, other cohort studies in Netherland and Ireland found no relationship between intake of carbohydrate, fat or protein during pregnancy and child obesity (Heppe et al., 2013; Murrin, Shrivastava, Kelleher, & Group, 2013). Interestingly, the Growing Up in Singapore Towards Healthy Outcomes study found that high intake of both carbohydrate and sugar during pregnancy was associated with higher BMI at 2-4 years of age (Chen et al., 2017). Collectively, data from these cohorts provide evidence for the role of sugar and other foods consumed during pregnancy with childhood obesity; nonetheless their results could be subject to residual and unmeasured confounding factors.

2.9.3. Breast-feeding and development of childhood obesity

The role of breast-feeding on childhood obesity has been of much interest. To date, there is only one RCT that evaluated the promotion of breast-feeding and in their findings they reported no association between the exclusiveness and duration of breast-feeding on childhood obesity at 6.5 years (Kramer et al., 2007). This is likely to be because the study was underpowered to detect the hypothesised effects (Ruckinger & von Kries, 2009).

There are currently more than 105 observational studies that evaluated whether breast-feeding could influence development of obesity in children, published between 1970 to 2014 in developing and developed countries. The study designs differed from cohort (prospective or retrospective) to cross-sectional and are of small to larger size. Exposure to breast-feeding were defined differently in the studies such as ever versus
never, exclusive breast-feeding for time points of <3, ≥4 and ≥6, 4-6 and 6-7 months versus never or less breast-fed. The outcome of interest was also reported for different age groups and adjusted for underlying variables mainly in cohort studies. Studies also used different criteria for measurement of overweight/obesity including Centers for Disease Control and Prevention (CDC) percentiles, International Obesity Task Force (IOTF) cutoffs, national reference data from Germany (BMI ≥97th percentile) as well as alternate BMI percentiles. The main weakness of studies that collected the data retrospectively was the length of recall of breast-feeding. The largest cohort conducted is the Scottish cohort that recruited 32,200 children between 1995-1996 and reported the obesity outcomes by 3-3.5 years (Armstrong & Reilly, 2002). It was reported that breast-fed infants had a lower rate of obesity (BMI≥98th percentile) (OR=0.70; 95% CI=0.61-0.80), adjusted for socioeconomic status, birth weight and sex. Given the large number of studies and inconsistency between the reported results on this topic, six systematic reviews with different inclusion criteria have been conducted published from 2004 to 2015. A recent overview of systematic reviews by Patro-Gołąb et al. (2016) has assessed the available evidence from the existing systematic reviews as well as two overviews of systematic reviews for exposure to breast-feeding on childhood BMI and obesity. The reviewers appraised the quality of systematic reviews included in their overview as high for one review, as low in one review with the remainder of medium quality. Overall, this evaluation of the current evidence, based on high-quality studies, indicates that ever breast-feeding leads to a modest reduction (13%) in childhood overweight and obesity, and a shorter duration is associated with a lesser protection. It should be noted however that the effect of residual confounders cannot be excluded. Interestingly, when only considering exclusive breast-feeding, no association was observed for later risk of childhood obesity. Collectively, the evidence-base highlights the impact of breast-feeding behaviours on developing obesity in children and could be introduced as an early preventive strategy.

The content of breast milk and its association with childhood obesity has also been assessed in studies. The Copenhagen prospective birth cohort study demonstrated that DHA levels in breast-milk, but not n-6/n-3 ratio, were directly associated with childhood BMI and fat mass from 2-7 years (Pedersen, Lauritzen, Brasholt, Buhl, & Bisgaard, 2012). Furthermore, it has been shown that breast milk of obese women
contains a higher level of n-6 to n-3 fatty acids suggesting that obesity is connected to an elevated pro-inflammatory fatty acid profile (Panagos et al., 2016).

Differences between breast-fed and formula-fed infants, in terms of developing obesity later in life, have been of much interest. Data from the Western Australian Pregnancy Cohort (Raine) Study in Perth indicated that a longer duration of breast-feeding reduced weight z-scores between birth and 1-year age (Oddy et al., 2014). Also, the age of introduction of formula was a significant risk factor for BMI trajectory from birth to 14 years with introducing formula at less than 6 months being associated with overweight or obese at 20 years. Studies have also found that breast milk in comparison to artificial formula milk contains lower protein and also stimulates less plasma insulin levels, and therefore leads to a reduced growth velocity in breast-fed infants (Oddy, 2012; Ziegler, 2006). Altogether, these data suggest the protective effect of a longer duration of breast-feeding against developing obesity later in life, so mothers might be advised to introduce formula at an older age.

2.9.4. Introduction of solid foods and development of childhood obesity

The effect of timing of introduction of complementary foods and types of food introduced is assessed in 30 observational studies, published “between 1978 to 2011”. Introduction of solids as the exposure in studies was defined differently for various age cut-offs including 2, 3, 4, 5 or 6 months, 4-5 or 4-6 months. Measures of weight and height/length were reported in all studies, whereas few measured BMI and z-scores as well as other growth and body composition measures such as skin-fold, circumference and ponderal index. Studies were also different for the duration of follow-up from less than 1 year to adulthood. The effect of confounders was considered in the majority of the studies; however there were wide variations between studies in the confounders measured such as maternal BMI and socio-economic status. Measurement of obesity outcome varied across studies. The Millennium Cohort Study in the UK is the largest study that investigated the effect of the timing of the introduction of solids as one of the risk factors for childhood obesity (Hawkins, Cole, Law, & Group, 2009). The study included a pool of 13,188 singleton children, born between 2000 and 2002 and defined childhood obesity by IOTF cut-offs for BMI. The findings of this study reported that introduction of solids at less than 4 months was associated with higher BMI at 3 years age (adjusted OR=1.12; 95%
CI=1.02-1.23). The association between timing and type of solid food introduction on obesity measures in children is investigated in six systematic reviews, published between 2001 to 2015. A recent overview of reviews assessed the available evidence from these systematic reviews and did not conduct meta-analysis (Patro-Goląb et al., 2016). In this overview, the quality of the conducted systematic reviews was rated as low for one and as medium for the remainder. This evaluation of the current evidence concluded that the effect of either overall timing of complementary feeding or the introduction before 15 weeks or four months on childhood obesity is not consistent. Furthermore, the available evidence does not suggest an association between types or patterns of complementary feeding as well as overall energy intake or sugar-sweetened beverage and early and late childhood obesity. In brief, the introduction of solids and its effect on obesity in children could be confounded by many underlying factors and needs to be considered in further studies.

2.10. Postnatal environmental factors and development of childhood obesity

The intestinal microbiota in infants has been suggested to play a significant role in childhood obesity. Two nested case-control studies in the prospective Finnish probiotic RCT, examining the compositional development of gut bacteria in infants, reported that a microbiota profile higher in bifidobacteria is protective against obesity in children whereas a microbiota in favour of *Staphylococcus aureus* is a risk factor for obesity later in life (Kalliomaki, Collado, Salminen, & Isolauri, 2008; Luoto et al., 2011). This finding suggests a role for gut microbiological environments on metabolic programming early in life.

Accelerated growth in infancy is also a risk factor for obesity in children and has been investigated in a number of observational studies. These studies are very heterogeneous in their design, sample and also definition and measurement of outcome using different criteria such as CDC percentiles, WHO charts and BMI percentiles. The Collaborative Perinatal Project, including 12 sites across the US, is the largest cohort that prospectively assessed the rapid rate of weight gain during the first 4 months of life with childhood overweight at 7 years age (Stettler, Zemel, Kumanyika, & Stallings, 2002). Adjusted analysis for several confounders showed an
association between higher rapid weight gain in early life and being overweight at 7 years old (OR=1.38; 95% CI=1.32-1.44). A systematic review including only cohort studies was conducted to investigate the evidence on this topic. A total of 10 cohorts were included, conducted across Europe, the US and the Seychelles (Druet et al., 2012). Using an external reference, weight SD scores at birth and 1-year age were estimated for each included child. Adjusted individual-level meta-analysis for sex, age and birth weight indicated that for each unit rise in weight SD scores from birth to 1-year of age, the risk of childhood obesity was doubled (OR=1.97; 95% CI=1.83-2.12) and also a higher risk was found for adult obesity (OR=1.23; 95% CI=1.16-1.30). Furthermore, a recent synthesis of 45 observational studies indicated that higher weight-for-length in the first two years of life was reported as a significant risk factor for childhood obesity in all studies (Woo Baidal et al., 2016). To summarise, the current evidence suggests that infant growth patterns in early life could be considered as an early preventive strategy for childhood obesity. The underlying mechanisms and potential confounders need to be addressed in further research.

The duration of sleep in infancy as a risk factor for childhood obesity is investigated in 38 observational studies. Studies were of different designs, mainly cross-sectional and published between 1990 to 2009 involving small to larger populations. Short duration of sleep, as the exposure was defined and measured differently in the conducted studies and obesity as the outcome measure, was also assessed using various criteria. Moreover, a range of different variables have been considered as confounders in studies. The largest birth cohort study was the Avon longitudinal study of parents and children in the UK that followed up 8,234 children by 7 years of age (Reilly et al., 2005). Short sleep duration (<10.5 hours) at age 3 years was reported as a risk factor for higher BMI, defined as ≥95th percentile relative to reference data for the UK population in 1990 (OR=1.45; 95% CI=1.10-1.89). Given the large heterogeneity between studies, a systematic review has assessed the evidence from current literature. This review only included longitudinal studies since the associations between sleep duration and developing obesity have been controlled for potential confounders more rigorously in prospective cohorts compared to cross-sectional studies. The evidence from 7 included studies was consistent and reported an inverse relation between the duration of sleep and later obesity in children (Magee & Hale, 2012). More recent data from the KOALA birth cohort study, including 2,322
children born between 2001 to 2003 in the Netherlands, reported that a longer nighttime sleep was associated with lower BMI or overweight by age 9 years, but not the daytime sleep (Bolijn, Gubbels, Sleddens, Kremers, & Thijs, 2016). Altogether, the current evidence suggests a role for short sleep duration and developing childhood obesity; however there are wide inconsistencies between studies for definition and measurement of exposure and outcomes as well as controlling for confounding factors. Experimental research may provide elucidating answers for the underlying mechanisms.

2.11. Prenatal nutritional approaches for prevention of childhood obesity

2.11.1. Nutritional interventions during pregnancy
A number of prenatal nutritional approaches have been introduced for prevention of obesity in infants. To avoid bias caused by selecting specific dietary interventions, all interventions were included in the review i.e. probiotics, fatty acids, diet-related and lifestyle approaches.

Evidence from experimental studies suggest that throughout pregnancy, both the fatty acid composition in maternal diet, specifically a ratio of n-6/n-3 fatty acid (Massiera et al., 2003), and also the amount of fat consumed (Armitage, Taylor, & Poston, 2005), are key elements for adipose tissue growth in the offspring. This finding could have an impact on the adipocyte number and fat cell size in infants since human adipose tissue starts to develop in the second trimester of pregnancy (Ailhaud & Hauner, 2004). It seems that fat cells acquired at an early stage in life determine the level of fully differentiated adipocytes later in life, with only approximately 10% of fat cells being replaced across all ages and at all levels of BMI (Spalding, Arner, & Westermark, 2008). Animal studies also indicated that AA, the major n-6 PUFA, may have a stimulatory effect on fat cell development, whereas DHA and EPA, the n-3 LCPUFA, counteract this process (Muhlhausler & Ailhaud, 2013). The anti-obesity effects of the latter are well recognised at decreased levels of adipose cells in tissue as well as lipid synthesis (Flachs, Rossmeisl, Bryhn, & Kopecky, 2009). Over recent decades, the dominance of n-6 fatty acids in diets, particularly in industrialised
countries, provides an indirect evidence that the n-6/n-3 fatty acid ratio may have a potential causal association with adipose tissue development during critical early phases of life (Ailhaud, Massiera, & Weill, 2006; Muhlhausler & Ailhaud, 2013). Findings from observational studies, however, do not support an association between n-6 and n-3 LCPUFA levels in maternal or cord blood and adiposity-related outcomes in offspring (de Vries et al., 2014; Donahue et al., 2011; Moon et al., 2013), as their results could be influenced by many underlying factors. Collectively these data suggest that maternal intake of n-3 fatty acids during pregnancy could be a good approach for early prevention of obesity later in life.

Experimental models have revealed that obesity is associated with altered gut microbial composition (Ley et al., 2005) and observational studies have shown that the gut microbiota of obese individuals is altered or less diverse compared to non-obese subjects (Ley, Turnbaugh, Klein, & Gordon, 2006; Turnbaugh et al., 2009). Also, key results from animal models reveal that the composition of gut microbiota modulates immune programming and can reduce the risk of obesity and metabolic diseases (Renz, et al., 2012; Turnbaugh et al., 2006). The development of gut colonisation occurs early in life since the foetus is initially exposed to maternal microbiota during pregnancy and delivery. Therefore, modification of gut microbiota in pregnant women with probiotics is proposed as a promising approach in reducing the risk of childhood obesity. Probiotics, as living microorganisms, can exert health benefits beyond inherent general nutrition and provide a safe microbial stimulus as part of the natural gut flora (Borriello et al., 2003; Guarner & Schaafsma, 1998). These characteristics make them a novel dietary choice in pregnancy.

2.11.2. Diet-related and lifestyle interventions during pregnancy

As discussed in section 2.9.1, an unfavourable intrauterine environment in pre-pregnancy caused by maternal obesity, excess GWG and GDM are proposed as potential risk factors in the development of obesity in offspring and thus identifying preventive approaches are of utmost importance. In its revised guidelines, the Institute of Medicine (IOM) has defined lower GWG targets during pregnancy for obese women at pre-pregnancy (Institute of Medicine and National Research Council, 2009) with the aim that defined GWG ranges could prevent short and long-term health outcomes in mother and child. Evidence also reveals that excess maternal weight gain
during pregnancy, beyond the recommended level, occurs in 53% of pregnant women already defined as exceeding normal weight gain (Simas et al., 2011). Since the excess nutrients required during pregnancy are shared between the mother and her foetus, early prevention strategies propose a window of opportunity for both monitoring weight in pregnant women and early prevention of obesity in infants. In this context, antenatal dietary interventions focused on the quality of the diet, and lifestyle advice involving physical activities, or a combination of both approaches are promising strategies for early prevention of childhood obesity.

2.12. Summary of the literature review for obesity

Obesity as a disease cannot be explained through genetics alone. It is clear that environmental factors play a significant role in the development of obesity early in life and thus are largely modifiable. Effective treatments for obesity are limited, and therefore environmental and nutritional approaches at early stages of life i.e. during pre-pregnancy and pregnancy could introduce an excellent opportunity to minimise susceptibility to lifelong obesity.
Chapter 3: The effectiveness of maternal nutritional/dietary interventions during pregnancy and the risk of developing allergic disorders in the offspring: systematic reviews and meta-analyses

3.1. Overview of the chapter

This chapter presents systematic reviews of literature describing the most recent available evidence from RCTs on the effectiveness of maternal dietary interventions during pregnancy for the prevention of allergic diseases in offspring. Dietary interventions explored include Pro/Prebiotics, Fatty Acids, Food Interventions and Vitamins/Supplements. The methodology section outlines: how the comprehensive literature search was conducted, the eligibility criteria used to assess studies against the defined inclusion criteria, and the techniques used for assessing the quality of included studies. The results section is divided by intervention type and by the Risk of Bias (ROB) assessment. The data is then presented in a descriptive manner. Where possible, the effect of outcomes have been presented using meta-analyses and where not possible, through narrative description. The discussion section summarises the findings from this systematic review, structured for each intervention type, and makes comparisons with the current literature and defines areas for further research.

3.2. Objectives

To conduct systematic reviews assessing the effectiveness of maternal dietary interventions during pregnancy for prevention of allergic disorders in the offspring

3.3. Methods and protocol for the systematic reviews

A protocol was developed adopting the approach of a Cochrane Systematic Review and finalised on 5th January 2015 (appendix 3.1) for the conduct of this systematic review, published in PROSPERO and accessible via the web-link below:

(http://www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42015024397)
3.3.1. Criteria for considering studies for review

3.3.1.1. Types of studies

Only RCTs including cluster randomised controlled trials and quasi-randomised controlled trials were included. The review considered studies, which documented clinical outcome data and had a follow-up period of at-least one month postnatally.

Systematic reviews, editorials, discussion papers, reports, case studies, case series, non-controlled before and after studies and animal studies were excluded.

3.3.1.2. Types of participants

Pregnant women from across the world selected from the general population and their offspring were considered as the target group in this systematic review. Studies conducted in high risk populations were not excluded.

3.3.1.3. Types of interventions

Studies reporting one or more of the following interventions during pregnancy were included:

   a. Pro/Prebiotic supplementation  
   b. Fatty acid supplementation  
   c. Food-based dietary advice (promoting a healthy diet) or food-based nutrient interventions  
   d. Vitamin/Multivitamin, supplementation and minerals (will be mentioned as Vitamins hereafter)

Trials were also included if the intervention(s) had been extended after pregnancy either in breast-feeding mothers, the infants or both. Studies within each of the above mentioned interventions were grouped under one umbrella intervention i.e. any pro/prebiotics, fatty acids, food intervention and vitamins, regardless of their specific applied intervention.

3.3.1.4. Outcomes of interest

Trials were included if they had reported clinical outcomes of allergy, either as a primary or secondary endpoint, in the offspring from infancy to adulthood. The primary and secondary outcome measures were defined as allergic diseases including
infantile wheeze. Where possible, outcomes that had utilised validated questionnaires were considered.

3.3.2. Search strategy for identification of studies

A comprehensive search strategy, including all the relevant synonyms for the main concepts, was developed covering the main bibliographic databases (see appendix 3.2).

3.3.2.1. Electronic searches

Trials were identified through systematic searches within three main electronic databases, as advised by the Cochrane collaboration (Lefebvre, Manheimer, & Glanville, 2011):

a. Cochrane Library (current issue) including:
   - Cochrane Database of Systematic Reviews (CDSR)
   - CENTRAL (trials)
   - Database of Abstracts of Reviews of Effect (DARE)

b. MEDLINE (EBSCOhost)

c. SCOPUS

When searching MEDLINE, the subject-specific terms were combined with the Cochrane Highly Sensitive Search Strategy for identifying randomised trials in MEDLINE: sensitivity-maximising version (Lefebvre, et al., 2011). We adapted the preliminary search strategy for MEDLINE (EBSCOhost) for use in the other databases when relevant. All databases were searched from their inception date to the end December 2014 (appendix 3.2).

In addition a number of supplementary searches were carried out as listed below:

a. ISI Web of Science (Thomson Web of Knowledge) (conference proceeding)

b. Clinicaltrials.gov

c. WHO International Clinical Trials Registry Platform (ICTRP)

d. E-theses Online Services (ETHoS )

The clinical trials registry and WHO platform were searched for ongoing and recently completed trials. Conference proceedings were identified through the ISI Web of Science and, to retrieve theses the British Library ETHoS was searched (Green & Higgins, 2011). No language or publication status restrictions were imposed.
3.3.2.2. Searching other sources of evidence

References of all identified studies and key systematic reviews in this area were checked for potentially relevant studies not identified by the above search strategy. There have been a number of systematic reviews that have evaluated the use of prenatal and/or postnatal probiotics as well as fatty acids for prevention of allergic diseases in offspring, with only one systematic review assessing the effectiveness of food interventions in this area. It is worth noting that there are no systematic reviews investigating the effectiveness of vitamin supplementations in pregnant women for prevention of allergies in offspring.

Systematic reviews of probiotics interventions have mostly investigated individual outcomes, mainly respiratory diseases or skin-related disorders (Azad et al., 2013; Betsi, Papadavid, & Falagas, 2008; Dang et al., 2013; Doege et al., 2012; Elazab et al., 2013; Lee, Seto, & Bielory, 2008; Pelucchi et al., 2012; Tang, Lahtinen, & Boyle, 2010) with one review assessing food allergy and sensitisation (Zhang et al., 2016). Of these, the review conducted by Doege et al., (2012) evaluated the impact of supplementation with probiotics on atopic eczema only for interventions introduced during pregnancy, whereas all the other reviews assessed the effectiveness of probiotics supplementation that commenced either prenatally, prenatal and continued postnatal or only postnatal. There are also two systematic reviews for fatty acids interventions in pregnant women. The review by Kremmyda et al., (2011) has narratively described the studies that supplemented women with n-3 Long Chain Poly Unsaturated Fatty Acids (n-3 LCPUFA) either prenatally only or prenatally continued postnatal for prevention of allergies in children; no meta-analyses were conducted. The other review has measured specific outcomes of asthma and atopic dermatitis in studies supplementing fatty acids prenatally, and conducted meta-analyses (Klemens, Berman, & Mozurkewich, 2011). Furthermore, the only review of food-based interventions during pregnancy has reported maternal and infant outcomes at birth (gestational weight gain, preterm birth, cord blood IgE) as well as childhood allergic outcomes measured at different time points (Kramer & Kakuma, 2012).

The main purpose of the current systematic review was to establish the most up to date evidence from RCTs, which have introduced the dietary intervention only
throughout pregnancy with a minimum follow-up of one month after birth. We have also aimed to investigate a comprehensive range of allergic outcomes presented in the trials. After the start date of the current systematic review, four reviews on the effectiveness of probiotics (Cuello-García et al., 2015; Zuccotti et al., 2015) and fatty acids (Best, Gold, Kennedy, Martin, & Makrides, 2016; Gunaratne, Makrides, & Collins, 2015) for prevention of multiple allergic outcomes in children have been published. Nevertheless, all these reviews either had different inclusion criteria and involved limited searching or considered different allergic outcomes.

The uniqueness of present systematic review in comparison to the previously conducted reviews is that we have looked specifically at the dietary interventions that started during pregnancy. This allowed us to investigate the evidence of whether the early commencement of dietary supplementations in pregnant women prevents childhood allergic outcomes. We have also included the most up-to-date follow-up data from the included trials in the meta-analyses.

3.3.3. Data collection and analysis

3.3.3.1. Selection of studies
The main reviewer (Mariam Vahdaninia, MV) initially screened all the search results against the eligibility criteria and all those clearly irrelevant, were excluded from further consideration. Thereafter, a tailored eligibility form was devised and used by MV to appraise the retrieved studies, abstract and full text, for relevance against the full inclusion criteria (appendix 3.3). Where there was uncertainty about inclusion of a particular study, other members of the review team were consulted and a consensus was reached about the study eligibility. All the included studies were discussed and approved by the review team and a Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) flowchart was developed. EndNote Reference Manager software was used to manage and record references.

Studies that did not meet the eligibility criteria are detailed in the table of characteristics of excluded studies (appendix 3.4).
3.3.3.2. Data extraction and management

EPPI Reviewer 4 (ER4) software was employed to manage the systematic review process. A detailed tailored data extraction tool was developed by MV and piloted on two of the included studies. Changes were made as appropriate and the final draft of the data extraction tool was discussed and agreed within the supervisory team (appendix 3.5). The main reviewer (MV) extracted the following baseline and detailed study characteristics from the included studies:

a. Study details: country, recruitment period, setting, ethics, informed consent and funding body
b. Trial type: details of trial design, number & name of study groups/arms, intention to treat analysis (ITT)
c. Study sample: comparability of groups, women’s age, risk of atopy in the studied sample, number at randomisation, number at follow-up, number of missing participants, reasons for missing, time points measured, length of follow-up
d. Intervention/comparison: detailed information about type of pro and prebiotics/fatty acids/vitamins/food avoidance, timing in pregnancy, mode of intervention delivery during pregnancy and/or infancy, total duration of intervention, side effects and detailed information about comparison used e.g. type, mode of delivery
e. Reported outcomes: all the reported clinical allergic end points, either as primary or secondary outcome, with the relevant definition e.g. point and or cumulative prevalence, crude or adjusted, combination of some outcomes
f. Diagnosis method: all methods defined for measuring the reported outcomes, e.g. questionnaires, clinical examinations and/or laboratory test(s)

All relevant data was extracted by MV. For studies with more than one control group, only the data for the placebo arm were extracted as a comparator.

Throughout the data extraction process, any disagreements about the interventions and outcomes were discussed and resolved within the review team. There was no blinding of the authors’ names, institutions, journals or the outcomes of the trials during the process. Ten percent of all the extracted data was randomly selected and
double checked by a second reviewer (Heather Mackenzie, HM) for accuracy against
the trial reports. Data was presented descriptively in tables and where possible, data
were combined and meta-analyses performed by utilising ER4, following the methods
described in the Cochrane Handbook of Systematic Reviews of Interventions (Deeks,
Higgins, & Altman, 2011), further details of which are provided below.

3.3.4. Assessment of risk of bias in included studies

The quality of each included trial was assessed by MV, using the risk of bias tool
described in the Cochrane Handbook for Systematic Reviews for Interventions
(Higgins, Altman, & Sterne, 2011). Risk of bias was considered according to the
following domains:

a. Random sequence generation: was the allocation sequence adequately
generated?
b. Allocation concealment: was allocation adequately concealed?
c. Blinding of participants and personnel: was knowledge of the allocated
intervention adequately prevented throughout the study i.e. blinded?
d. Blinding of outcome assessments: was knowledge of the outcome assessment
adequately prevented i.e. blinded?
e. Incomplete outcome data: were incomplete outcome data adequately
addressed for every outcome?
f. Selective outcome reporting: were reports of the study free of selective
outcome reporting?
g. Other biases: was the study free from any other problems that could put it at
risk of bias e.g. comparability of control group at entry, industry funding?

Based on these criteria, each study was scored using a three-point scale: low risk of
bias, high risk of bias, unclear. Disagreements resolved with the review team which
consisted of MV & HM and if required, by consensus with a third reviewer (Tara
Dean, TD). The risk of bias results were presented in risk of bias tables and were
taken into account when considering treatment effect.

3.3.4.1. Measurement of treatment effect

Dichotomous data was analysed as risk ratios or relative risk (RR) with 95%
confidence intervals and continuous data as mean difference or standardised mean
difference, with 95% confidence intervals (CI).
3.3.4.2. Unit of analysis issues

In trials with more than one intervention arm, multiple pairwise comparisons of intervention groups versus comparator were avoided. Therefore, data from different intervention arms were pooled for an overall comparison with the control or placebo arm. The weight assigned to the control group was considered as the total number of participants in the comparator group versus the total number of participants in the combined intervention arms (Deeks, et al., 2011). Similarly, in studies with more than one comparator arm, defined as placebo and no treatment, only data from the placebo arm was compared with the intervention arm.

3.3.4.3. Handling missing data

All of the relevant reported information for the number of missing participants was extracted and, if undocumented, this was incorporated into the assessment of risk of bias.

In most of the studies, the missing data was reported to be as a result of loss to follow-up and was commonly stated as being normally distributed across the study arms. As only a small number of studies had reported the results based on ITT, the per-protocol analysis was used for the systematic review. Studies that performed ITT analysis are identified in the table of characteristics of included studies (Table 3.2).

3.3.4.4. Assessment of heterogeneity

To measure statistical heterogeneity between effect sizes of included studies, within each umbrella intervention group and for each allergy outcome separately, we used visual inspection of forest plots and also, the $\chi^2$ test for heterogeneity with a P Value <0.05 (Deeks, Altman, & Bradburn, 2001). The $I^2$ statistics were used to quantify the amount of possible variability in effect estimates that is due to heterogeneity rather than chance ($I^2 >30\%$ moderate heterogeneity, $I^2 \geq 75\%$ considerable heterogeneity). Moreover, studies with a similar comparator, within each umbrella intervention group, were grouped to run meta-analyses.

With regards to clinical heterogeneity, two key issues were considered. Firstly, data for all reported allergic outcomes from the included trials were extracted, crude or adjusted. The following outcomes were considered for this systematic review (Table
3.1). Only crude reported outcomes and also, the longest available follow-up measures were selected for this systematic review and entered in meta-analyses.

**Table 3.1. List of the outcomes of interest for this systematic review**

<table>
<thead>
<tr>
<th>Main Allergic outcomes</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any allergic disease(s)</td>
<td>Latest available follow-up data</td>
</tr>
<tr>
<td>Wheeze</td>
<td>Latest available follow-up data</td>
</tr>
<tr>
<td>Eczema</td>
<td>Latest available follow-up data, includes atopic dermatitis</td>
</tr>
<tr>
<td>Asthma</td>
<td>Latest available follow-up data</td>
</tr>
<tr>
<td>Rhinitis</td>
<td>Latest available follow-up data, excludes rhinoconjunctivitis</td>
</tr>
<tr>
<td>Food Allergy</td>
<td>Latest available follow-up data (may include various methods of measurement)</td>
</tr>
<tr>
<td>Anaphylaxis</td>
<td>Presented narratively as reported in one study only</td>
</tr>
<tr>
<td>Angioedema</td>
<td>Presented narratively as reported in one study only</td>
</tr>
<tr>
<td><strong>SPT and IgE tests</strong></td>
<td></td>
</tr>
<tr>
<td>SPT (any positive)</td>
<td>Either latest available follow-up data or earlier reports</td>
</tr>
<tr>
<td>Specific IgE</td>
<td>Either latest available follow-up data or earlier reports</td>
</tr>
</tbody>
</table>

* Either point or cumulative prevalence ** Skin Prick Test *** Immunoglobulin E

Secondly, as there were a number of published systematic reviews on the effectiveness of probiotics, fatty acids and food interventions during pregnancy for prevention of allergic disorders in offspring, a broad definition of allergic outcomes was employed in meta-analyses, which allowed for clinical heterogeneity in all the included studies. As mentioned in section 3.3.2.2, the current body of evidence from systematic reviews has investigated the effectiveness of prenatal and/or postnatal probiotics for prevention of selected allergic outcomes in children. These reviews also scrutinised the effectiveness of probiotics in sub-group analyses for the type of participants (mother only, both mother & infant, infant only), timing of intervention, probiotic dose and organism, risk of developing allergy in infant, diagnosis method of allergic diseases, duration of follow-up, geographical area and risk of bias (Azad et al., 2013; Dang et al., 2013; Doege et al., 2012; Elazab et al., 2013; Pelucchi et al., 2012; Zhang et al., 2016). The most recent review by Cuello-Garcia et al., 2015 looked at all allergic outcomes as the probiotic intervention was applied time points: during pregnancy (trans-placental), in breast-feeding mothers (indirect evidence), in infants by oral preparation or milk formula (direct evidence) or a combined approach for timing i.e. during pregnancy and continued after birth. For fatty acid interventions, the Gunaratne et al. (2015) review included all allergic outcomes and measured the evidence only from trials supplementing n-3 LCPUFA as the intervention, either in pregnancy or lactation, and reported detailed outcomes of IgE and non-IgE-mediated
allergic disorders including sensitivity analyses. The systematic review by Best et al. (2016) investigated the evidence from observational and RCTs separately that only used n-3 LCPUFA supplements during pregnancy and reported IgE-mediated allergic outcomes in the offspring. The only systematic review for food-based intervention trials by Kramer and Kakuma (2012) also conducted sub-group analyses for different follow-up time points and specific food allergens.

Based on the above-mentioned, broad definitions of allergic outcomes were introduced into the conducted meta-analyses in current systematic review and sub-group analyses were avoided for the criteria as defined below:

a. Duration of intervention; prenatal only or continued postnatal

b. Specific strain/type of pro/prebiotics, fatty acids, food-based interventions

c. Method of diagnosis

d. Length of follow-up

e. Selected/unselected sample

f. Dosage/frequency/delivery mode of intervention

g. Study location & setting

j. Where reported, cumulative prevalence/incidence are included in meta-analyses and if not reported, point prevalence(s) are considered

k. Outcomes solely based on parental report, which applies to the broadest definition of the outcome, have been reported descriptively.

It was believed that this approach could add to the current evidence base and offer a view for the overall benefit of prenatal dietary interventions for prevention of childhood allergies.

3.3.4.5. Assessment of reporting biases

Every effort was made to identify unpublished studies through searching abstracts and ongoing trials databases as described in section 3.3.2. Publication bias was assessed using funnel plots, when there were ≥10 studies included in the meta-analysis because the power of the test would be high enough to distinguish chance from real asymmetry (Egger, Davey Smith, Schneider, & Minder, 1997; Sterne, Egger, & Smith, 2001). The asymmetry was assessed visually in the plots produced using ER4,
and no formal statistical tests were conducted. The funnel plots were helpful to explore possible small study biases for some of the primary outcomes.

### 3.3.4.6. Data synthesis

All meta-analyses were conducted using a random-effects model in view of the clinical and methodological differences between included studies. Dichotomous data were entered as events and the number of participants. Data were pooled using random-effects model only where heterogeneity was reported as $\leq 75\%$ and also relative risk (RR) was reported as a statistical choice in conducting the meta-analyses, as it is easy to interpret (Deeks et al., 2011).

### 3.4. Changes to the protocol

There were four changes made to the protocol whilst conducting the review. Firstly, in the protocol for this review, it was initially stated that in trials with multiple intervention groups, data for the control group would be used for each intervention group comparison and the weight assigned to the control group would be reduced by dividing the number of participants in the control group by the number of intervention groups. However for more clarity, and as recommended by Cochrane Collaboration (Deeks et al., 2011), it was decided to pool the data for each intervention arm in these trials and make the comparison for the pooled intervention arm versus the control group. So, the weight assigned to the intervention group was considered as the total number of participants in intervention arms divided by the number of participants in the control group.

Secondly, it was intended to use the per-protocol analysis for conducting all the meta-analyses; however this was not possible for some papers as ITT was the only available data, and these reported data were used in performing meta-analyses.

Thirdly, it was planned to narratively describe study results when outcome data was solely based on parental reporting. However for one of the papers which utilised food intervention, the outcomes “atopic eczema, with parental opinion” as well as ‘food allergy, parental report’ are entered in meta-analysis, as these were the only specific reported outcome in the study.
Lastly, as a broad definition of allergic disorders has been considered in this review, in terms of statistical and clinical heterogeneity, we did not plan to conduct any detailed sub-group and/or sensitivity analyses for pre/probiotic, fatty acid and food intervention studies. However where required, some sub-group analyses have been performed and the explanation for this has been provided in the accompanying text. It is worth mentioning, since this is the first systematic review for vitamin supplementations on prevention of allergic diseases in the offspring, we have conducted sub-group analyses for this dietary group. However it was not possible to conduct sensitivity analyses since there were a limited number of studies that could contribute in the meta-analyses in the dietary group as vitamins.

3.5. Results

3.5.1. Studies identified through searches and total included studies

Searches of electronic databases were initially carried out between November and December 2014 and updated on 31st January 2016. The searches yielded a total of 3,271 references and as specified in the protocol, removal of duplicates and non-relevant studies (1,384) from the 1,489 included studies for screening the title and abstract left 105 papers for further consideration. Of the remaining 105 references, 46 were excluded after closer inspection showed that they had either reported an inappropriate study outcome or had inappropriate study design/participants and in the case of one paper, that it was fabricated data. Of the studies excluded due to inappropriate design at this stage, one was in Spanish and a native Spanish speaker was consulted to find out whether the paper met the inclusion criteria for this systematic review. As the study was a non-randomised trial, with food avoidance as the intervention, it was excluded (listed in Appendix 3.4).

Full-text screening of the remaining 59 papers showed that they were either a study protocol (n=2) or earlier published reports of the included studies (n=29). These earlier reports, hereafter referred to ‘linked records/companion papers’, were used to extract any relevant data of the initial trial, if required. Also, supplementary searches from other databases resulted in two papers. As a result, a total number of 32 studies were included in the systematic reviews. The PRISMA flow diagram is shown in Figure 3.1.
To supplement the electronic searching, the reference lists of papers selected for full text screening as well as key systematic reviews on the topic were scrutinised to identify any further studies.

Earliest reports/papers of the trials included in the systematic reviews and were used for data extraction if required.

**Figure 3.1. PRISMA flowchart for the literature search strategy-Allergy outcomes**

The list of the included studies that met the inclusion criteria for this systematic review, by intervention type, is shown in Table 3.2. All trials were in English and had been carried out in various countries across the world. The descriptive findings, risk of bias assessment and effects of interventions are structured by intervention in the following sections.
Table 3.2. List of the included trials

<table>
<thead>
<tr>
<th>Probiotic Interventions</th>
<th>AOD</th>
<th>ITT</th>
<th>Fatty Acid Interventions</th>
<th>AOD</th>
<th>ITT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kalliomäki 2003</td>
<td>✓</td>
<td>NI</td>
<td>Dunstan 2003</td>
<td>✓</td>
<td>NI</td>
</tr>
<tr>
<td>Kalliomäki 2007</td>
<td>✓</td>
<td>NI</td>
<td>Olsen 2008</td>
<td>✓</td>
<td>-</td>
</tr>
<tr>
<td>Huurre 2008</td>
<td>✓</td>
<td>NI</td>
<td>Linnamaa 2010</td>
<td>✓</td>
<td>-</td>
</tr>
<tr>
<td>Kopp 2008</td>
<td>✓</td>
<td>-</td>
<td>Furuhjelm 2011</td>
<td>✓</td>
<td>-</td>
</tr>
<tr>
<td>Niers 2009</td>
<td>✓</td>
<td>-</td>
<td>Noakes 2012</td>
<td>✓</td>
<td>NI</td>
</tr>
<tr>
<td>Kuitunen 2009</td>
<td>✓</td>
<td>-</td>
<td>Palmer 2013</td>
<td>-</td>
<td>✓</td>
</tr>
<tr>
<td>Kim 2010</td>
<td>✓</td>
<td>-</td>
<td>Escamilla-Nunez 2014</td>
<td>✓</td>
<td>-</td>
</tr>
<tr>
<td>Dotterud 2010</td>
<td>✓</td>
<td>✓</td>
<td>Food Interventions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boyl 2011</td>
<td>✓</td>
<td>✓</td>
<td>Lilja 1989</td>
<td>✓</td>
<td>-</td>
</tr>
<tr>
<td>Rautava 2012</td>
<td>✓</td>
<td>✓</td>
<td>Zeiger 1992</td>
<td>✓</td>
<td>-</td>
</tr>
<tr>
<td>Ou 2012</td>
<td>✓</td>
<td>✓</td>
<td>Fälth-Magnusson 1992</td>
<td>✓</td>
<td>-</td>
</tr>
<tr>
<td>Abrahamsson 2013</td>
<td>✓</td>
<td>NI</td>
<td>Lovegrove 1994</td>
<td>✓</td>
<td>-</td>
</tr>
<tr>
<td>Wickens 2013</td>
<td>✓</td>
<td>✓</td>
<td>Vitamin Interventions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allen 2014</td>
<td>✓</td>
<td>✓</td>
<td>Greenough 2010</td>
<td>✓</td>
<td>Yes</td>
</tr>
<tr>
<td>Gorissen 2014</td>
<td>✓</td>
<td>✓</td>
<td>Goldring 2013</td>
<td>✓</td>
<td>No</td>
</tr>
<tr>
<td>Simpson 2015</td>
<td>✓</td>
<td>✓</td>
<td>McEvoy 2014</td>
<td>✓</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Chawes 2016</td>
<td>-</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Litonjua 2016</td>
<td>-</td>
<td>Yes</td>
</tr>
</tbody>
</table>

*For studies with data available on both observed and ITT analysis, the observed data are included in meta-analysis.
Ante. AOD=Analysis on the Observed Data; ITT=Intention to Treat Analysis, some trials have conducted the ITT only for certain outcomes; NI=No Information

3.5.2. Presentation of the results

The Meta-analyses for assessing the effect of intervention are structured by the intervention type. Within each intervention category, studies are grouped and assessed for the clinical outcome(s) of interest for this systematic review. Detailed descriptions of the outcomes included in the meta-analyses are also presented. As described in section 3.3.4.4, meta-analyses were conducted using a random effect model and with the exception for vitamin studies, no detailed sub group analyses were conducted for the pro/prebiotics, fatty acids and food intervention dietary groups.
3.5.3. Description of included studies of maternal pro/prebiotic consumption during pregnancy and prevention of allergic diseases in the offspring

Of the 32 included trials, 16 examined the impact of probiotic interventions on the development of allergic diseases in offspring, including 3,567 children (this number also comprises the three earlier reports of the included trials). One study only examined the impact of a combination of probiotics and prebiotics on the development of allergic diseases in offspring (Kuitunen, Kukkonen, & Savilahti, 2009) while all other studies have used different strains of probiotics only. In the case of three trials, both the reports of an earlier as well as the latest follow-up data were included, since some of the outcomes of interest for this systematic review were reported only in the earlier published papers of these trials. These studies were as below:

i. Kalliomäki, Salminen, Poussa, & Isolauri, 2007 reported the longer follow-up of Kalliomaki, Salminen, Poussa, Arvilommi, & Isolauri, 2003
ii. Gorissen et al., 2014 reported the longer follow-up of Niers et al., 2009
iii. Simpson, Dotterud, Storrø, Johnsen, & Øien, 2015 reported the longer follow-up of Dotterud, Storrø, Johnsen, & Øien, 2010

The detailed characteristics of the included trials, their companion papers and study population are shown in Table 3.3. In total, four studies were conducted in Finland and the rest in Sweden, United Kingdom, Australia, Norway, Korea, Germany, Netherlands, New Zealand and Taiwan. All studies used placebo as their comparator/control.

The longest follow-up period was 7 years, in the study conducted by (Abrahamsson, Jakobsson, Bjorksten, Oldaeus, & Jenmalm, 2013), followed by 6 years in studies by (Gorissen et al., 2014; Kalliomäki, et al., 2007; Simpson, et al., 2015; Wickens et al., 2013). The largest study sample was reported in (Kuitunen, et al., 2009) with 1,223 mothers enrolled followed by Wickens et al. (2013) and (Allen et al., 2014) with 511 and 454 pregnant mothers at enrolment respectively. The smallest sample size was observed in studies conducted by (Kopp, Hennemuth, Heinzmann, & Urbanek, 2008) and (Kim et al., 2010) with 105 and 112 mothers randomised at recruitment.
respectively.

With the exception of two studies (Allen et al., 2014; Simpson, et al., 2015), the study sample was selected from the families with a reported history of atopic diseases.

The most frequently reported outcomes listed were: wheeze, eczema, asthma and positive SPT.

Compliance with the intervention was assessed by a variety of methods, including maternal interview or daily diaries, counting of unused supplements and faecal examination. Method of adherence to intervention was not reported in the studies conducted by (Huurre, Laitinen, Rautava, Korkeamäki, & Isolauri, 2008; Kalliomaki, et al., 2003; Kopp, Hennemuth, Heinzmann, & Urbanek, 2008; Ou et al., 2012; Rautava, Luoto, Salminen, & Isolauri, 2012).
<table>
<thead>
<tr>
<th>Primary article</th>
<th>Companion articles+</th>
<th>Country, enrolment period</th>
<th>Trial type</th>
<th>Study intervention &amp; comparator</th>
<th>No. of participants **</th>
<th>No. at last F-U***</th>
<th>Time points measured</th>
<th>Age at last F-U</th>
<th>Sample: high risk of Atopy</th>
<th>Outcomes reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Kalliomaki et al., 2003)</td>
<td>(Kalliomaki et al., 2001) (Rautava, Kalliomäki, &amp; Isolauri, 2002)</td>
<td>Finland 1997-98</td>
<td>PC-RCT</td>
<td>LGG Probiotics &amp; Placebo</td>
<td>159 mothers</td>
<td>107 (53 vs. 54)</td>
<td>1, 1.5, 2 &amp; 4yrs.</td>
<td>4yrs.</td>
<td>Yes</td>
<td>-Eczema -Asthma -SPT (any positive) -SPT (peanut) -SPT (CAT) -SPT (Cod) -SPT (grass) -SPT (Dog) -Food Allergy -Rhinitis -SPT (Birch) -SPT (Alder)</td>
</tr>
<tr>
<td>(Kalliomaki et al., 2007)</td>
<td>(Kalliomaki et al., 2001) (Rautava, et al., 2002)</td>
<td>Finland 1997-98</td>
<td>PC-RCT</td>
<td>LGG Probiotics &amp; Placebo</td>
<td>159 mothers</td>
<td>116 (53 vs. 62)</td>
<td>1, 1.5, 2, 4 &amp; 6yrs.</td>
<td>6yrs.</td>
<td>Yes</td>
<td>-Eczema -Asthma -SPT (any positive) -Rhinitis</td>
</tr>
<tr>
<td>(Huurre, et al., 2008)</td>
<td>(Piirainen, Isolauri, Lagstrom, &amp; Laitinen, 2006)</td>
<td>Finland 2002-unclear</td>
<td>PC-RCT</td>
<td>Mixed probiotics &amp; placebo</td>
<td>140 mothers</td>
<td>140 (72 vs. 68)</td>
<td>1,6 &amp; 12 months</td>
<td>1yr.</td>
<td>Yes</td>
<td>-Eczema -SPT (any positive)</td>
</tr>
<tr>
<td>(Kopp, et al., 2008)</td>
<td>(Kopp et al., 2008)</td>
<td>Germany 2002-04</td>
<td>PC-RCT</td>
<td>LGG &amp; Placebo</td>
<td>105 mothers</td>
<td>94 (50 vs. 44)</td>
<td>6,12 &amp; 24 months</td>
<td>2yrs.</td>
<td>Yes</td>
<td>-Wheeze -Eczema -Upper Respiratory Tract Infection -Any IgE</td>
</tr>
<tr>
<td>(Niers et al., 2009)</td>
<td>(Gorissen et al., 2014)</td>
<td>Netherlands 2004-05</td>
<td>PC-RCT</td>
<td>Mixed Probiotic bacteria &amp; placebo</td>
<td>156 mothers</td>
<td>98 (50 vs. 48)</td>
<td>3, 6, 12 &amp; 24months</td>
<td>2yrs.</td>
<td>Yes</td>
<td>-Eczema -SPT (any positive) -SPT (food) -Specific IgE -Sensitisation (Either positive SPT or/and sIgE &gt;0.35 IU/ml)</td>
</tr>
<tr>
<td>Primary article</td>
<td>Companion articles+</td>
<td>Country, enrolment period</td>
<td>Trial type*</td>
<td>Study intervention &amp; comparator</td>
<td>No. of participants **</td>
<td>No. at last F-U***</td>
<td>Time points measured</td>
<td>Age at last F-U</td>
<td>Sample: high risk of Atopy</td>
<td>Outcomes reported</td>
</tr>
<tr>
<td>------------------------------------------------------</td>
<td>---------------------</td>
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<tr>
<td>(Kuitunen, et al., 2009)</td>
<td>(Kukkonen, Nieminen, Poussa, Savilahti, &amp; Kuitunen, 2006) (Kukkonen et al., 2007) (Kukkonen et al., 2008)</td>
<td>Finland 2000-03</td>
<td>PC-RCT</td>
<td>Mixed Probiotic &amp; placebo</td>
<td>1,223 mothers</td>
<td>891 (445 vs. 446)</td>
<td>3, 6, 12, 24months &amp; 5yrs.</td>
<td>5yrs.</td>
<td>Yes</td>
<td>-SPT (Inhalation allergens) -Any IgE</td>
</tr>
<tr>
<td>(Kim et al., 2010)</td>
<td>None</td>
<td>Korea 2005-06</td>
<td>PC-RCT</td>
<td>Mixed Probiotics &amp; placebo</td>
<td>112 mothers</td>
<td>68 (33 vs. 35)</td>
<td>3, 6 &amp; 12 months</td>
<td>1yr.</td>
<td>Yes</td>
<td>-Eczema -IgE-associated eczema -Specific IgE (any food) -Any IgE -Probable Food Allergy (egg) -Probable food allergy (cow’s milk)</td>
</tr>
<tr>
<td>(Dotterud, et al., 2010)</td>
<td>(Simpson, et al., 2015)</td>
<td>Norway 2003-05</td>
<td>PC-RCT</td>
<td>Pro milk &amp; placebo milk</td>
<td>415 mothers</td>
<td>278 (138 vs. 140)</td>
<td>2yrs</td>
<td>2yrs.</td>
<td>No</td>
<td>-Eczema -Asthma -SPT (any positive) -Specific IgE -Allergic Rhinoconjunctivitis -Atopic sensitised</td>
</tr>
<tr>
<td>(Boyle et al., 2011)</td>
<td>(Boyle et al., 2008) (Lahtinen, Boyle, Kivivuori, Oppedisano,)</td>
<td>Australia 2006-08</td>
<td>PC-RCT</td>
<td>LGG &amp; placebo</td>
<td>250 mothers</td>
<td>212 (109 vs. 103)</td>
<td>3, 6 &amp; 12 months</td>
<td>1yr.</td>
<td>Yes</td>
<td>-Eczema -SPT (any positive) -SPT (egg) -SPT (peanut) -SPT (cows milk) -SPT (food) -SCORAD (0, 1-25,</td>
</tr>
<tr>
<td>Primary article</td>
<td>Companion articles+</td>
<td>Country, enrolment period</td>
<td>Trial type</td>
<td>Study intervention &amp; comparator</td>
<td>No. of participants **</td>
<td>No. at last F-U ***</td>
<td>Time points measured</td>
<td>Age at last F-U</td>
<td>Sample: high risk of Atopy</td>
<td>Outcomes reported</td>
</tr>
<tr>
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<tr>
<td>Smith, 2009</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>25-50, &gt;50) SPT (aeroallergen)</td>
</tr>
<tr>
<td>(Rautava, et al., 2012)</td>
<td>None</td>
<td>Finland 2005-09</td>
<td>PC-RCT</td>
<td>2 diff. mixed probiotic arms &amp; placebo</td>
<td>241 mothers</td>
<td>205 (73 vs. 70 vs. 62)</td>
<td>6, 12 &amp; 24 months</td>
<td>2yrs.</td>
<td>Yes</td>
<td>-Eczema -SPT (any positive)</td>
</tr>
<tr>
<td>(Ou et al., 2012)</td>
<td>Kuo 2012 (conference presentation)</td>
<td>Taiwan 2002-06</td>
<td>PC-RCT</td>
<td>LGG Probiotics &amp; placebo</td>
<td>191 mothers</td>
<td>128 (65 vs. 63)</td>
<td>6, 18 &amp; 36 months</td>
<td>3yrs.</td>
<td>Yes</td>
<td>-Any Allergic disease(s) -Wheeze -Eczema -Specific IgE -Sneezing and/or snuffling -Any IgE</td>
</tr>
<tr>
<td>(Wickens et al., 2013)</td>
<td>(Wickens et al., 2008) (Wickens et al., 2012) (Dekker et al., 2009) (S.L. Prescott et al., 2008)</td>
<td>New Zealand 2004-05</td>
<td>PC-RCT</td>
<td>2 diff mixed probiotic arms &amp; placebo</td>
<td>511 mothers</td>
<td>422(134 vs. 144 vs. 144)</td>
<td>2, 4 &amp; 6yrs.</td>
<td>6yrs.</td>
<td>Yes</td>
<td>-Wheeze -Eczema -Asthma -Allergic Rhinoconjunctivitis -SPT (any positive) -Specific IgE -SCORAD -Any IgE</td>
</tr>
<tr>
<td>(Allen et al., the UK)</td>
<td>Allen et al.,</td>
<td>the UK</td>
<td>PC-</td>
<td>Mixed probiotic &amp;</td>
<td>454</td>
<td>378 (187</td>
<td>6months &amp;</td>
<td>2yrs.</td>
<td>No</td>
<td>-Wheeze -Eczema (atopic)</td>
</tr>
<tr>
<td>Primary article</td>
<td>Companion articles+</td>
<td>Country, enrolment period</td>
<td>Trial type*</td>
<td>Study intervention &amp; comparator</td>
<td>No. of participants **</td>
<td>No. at last F-U***</td>
<td>Time points measured</td>
<td>Age at last F-U</td>
<td>Sample: high risk of Atopy</td>
<td>Outcomes reported</td>
</tr>
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<tr>
<td>2014)</td>
<td>2010)</td>
<td>2005-unclear</td>
<td>RCT</td>
<td>placebo</td>
<td>mother-infant dyads</td>
<td>vs. 191)</td>
<td>2yrs.</td>
<td></td>
<td></td>
<td>-Asthma -SPT (any positive) -SPT (egg) -SPT (HDM) -SPT (cow's milk) -SPT (CAT) -SPT (grass) -Food Allergy -Rhinitis -Cough -Chronic cough -Sneezing and/or snuffling</td>
</tr>
<tr>
<td>(Gorissen et al., 2014)</td>
<td>(Niers et al., 2009)</td>
<td>Netherlands 2004-05</td>
<td>PC-RCT</td>
<td>Mixed Probiotic &amp; placebo</td>
<td>156 mothers</td>
<td>83 (39 vs. 44)</td>
<td>3, 12 &amp; 24months, 6yrs.</td>
<td>6yrs.</td>
<td>Yes</td>
<td>-Eczema -Asthma -Food Allergy -Rhinitis</td>
</tr>
<tr>
<td>(Simpson, et al., 2015)</td>
<td>(Dotterud, et al., 2010)</td>
<td>Norway 2003-05</td>
<td>PC-RCT</td>
<td>Probiotic milk &amp; placebo milk</td>
<td>415 mothers</td>
<td>163 (81 vs. 82)</td>
<td>2 &amp; 6yrs.</td>
<td>6yrs.</td>
<td>No</td>
<td>-Eczema -Asthma -Allergic sensitisation (any SPT or Specific IgE) -Allergic Rhinoconjunctivitis -Wheeze -Lower respiratory tract infection</td>
</tr>
</tbody>
</table>

+Published data and conference presentations, No unique data were extracted from conference abstracts

*Placebo Controlled-Randomised Controlled Trial

**Indicates the number at randomisation, where recruitment has occurred prenatally

***Follow-up
Table 3.4 shows the details of the pro/prebiotic interventions and placebo used in included trials. In all studies the intervention and placebo groups had comparable baseline characteristics at recruitment. It is worth noting that the Abrahamsson et al. (2013) study reported higher antibiotic prescription during the first year of life in the intervention vs. placebo group (p=0.03).

Only one study administered probiotics solely during pregnancy (Boyle et al., 2011). In the remainder, the probiotic interventions were continued after pregnancy either in mothers only or with both mothers and their infants for a period of time (Table 3.4). The longest duration of intervention was 25 months (Wickens et al., 2013) and the shortest was 2-4 weeks (Boyle et al., 2011). In five studies, a single strain of probiotics was used (Abrahamsson et al., 2013; Boyl et al., 2011; Kalliomäki et al., 2003 & 2007; Kopp et al., 2008; Ou et al., 2012); whereas a mixed strain of probiotics was employed in the other included trials. For the purpose of this systematic review, all the probiotic intervention studies, whether they used mixed or single strains of probiotics, have been grouped together under one umbrella as “any probiotics”.

Two studies included two active intervention arms employing different mixed strains of probiotics in each intervention group and comparing these with a placebo (Rautava, et al., 2012; Wickens et al., 2013). For reporting purposes and as recommended by the Cochrane Handbook (Deeks et al., 2011), data from the two different active intervention arms were pooled when entered into meta-analysis.

Probiotic preparations included oil drops, capsules, milk and sachets. The study conducted by Huurre, et al., 2008 provided women with nutritional advice during pregnancy and breastfeeding as well as randomising them to receive mixed probiotics. The dietary counselling focused on the amount and type of fat and also the amount of fibre in their diet.
<table>
<thead>
<tr>
<th>Primary article</th>
<th>Comparable baseline characteristic</th>
<th>Participants receiving intervention</th>
<th>Timing of Intervention in pregnancy</th>
<th>Intake of Intervention from/until</th>
<th>Duration of intervention (months)**</th>
<th>Probiotic organism***</th>
<th>Placebo</th>
<th>Mode of delivery</th>
<th>Total daily dose (Colony Forming Units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Kalliomaki et al., 2003; Kalliomaki et al., 2007)</td>
<td>Yes</td>
<td>Prenatally and postnatally in mothers and infants</td>
<td>2–4wks before expected delivery</td>
<td>6 months in B-F mothers</td>
<td>6.5-7</td>
<td>LGG</td>
<td>Microcrystalline cellulose</td>
<td>Capsule</td>
<td>$1 \times 10^{10}$ CFU/day, daily</td>
</tr>
<tr>
<td>(Huurre, et al., 2008)</td>
<td>Yes</td>
<td>Prenatally and postnatally in mothers</td>
<td>From 1st trimester of pregnancy</td>
<td>9wks to 4months (exclusive BF)</td>
<td>10.5-11</td>
<td>LGG + BL Bb12</td>
<td>Microcrystalline cellulose &amp; dextrose anhydrates</td>
<td>Capsule</td>
<td>$1 \times 10^{9}$ CFU/day for each probiotic strain, daily</td>
</tr>
<tr>
<td>(Kopp, et al., 2008)</td>
<td>Yes</td>
<td>Prenatally and postnatally in mothers and infants</td>
<td>4–6wks before expected delivery</td>
<td>3 months</td>
<td>7-7.5</td>
<td>LGG</td>
<td>Microcrystalline cellulose</td>
<td>Capsule</td>
<td>$5 \times 10^{9}$ CFU/day Twice a day</td>
</tr>
<tr>
<td>(Niers et al., 2009; Gorissen et al., 2014)</td>
<td>Yes</td>
<td>Prenatally and postnatally in mothers and infants</td>
<td>During the last 6wks of pregnancy</td>
<td>34wks to 12mon postnatal</td>
<td>13.5</td>
<td>BB W23 + BL W52 + LL W58 Carrier of the probiotic product i.e. rice starch &amp; maltodextran</td>
<td>Sachets</td>
<td>$1 \times 10^{10}$ CFU/day for each probiotic strain, 3g daily</td>
<td></td>
</tr>
<tr>
<td>(Kuitunen, et al., 2009)</td>
<td>Yes</td>
<td>Prenatally and postnatally in infants</td>
<td>From 36wks of gestation</td>
<td>6 months postnatal</td>
<td>6.5-7</td>
<td>LC705 + LC705 +bb99 + Pf</td>
<td>Microcrystalline cellulose</td>
<td>Capsule</td>
<td>$5 \times 10^{9}, 5 \times 10^{8}, 2 \times 10^{8}, 2 \times 10^{7}$ CFU/day, twice a day</td>
</tr>
<tr>
<td>(Kim et al., 2010)</td>
<td>Yes</td>
<td>Prenatally and postnatally in mothers and infants</td>
<td>From 8wks before the expected delivery</td>
<td>3 months after delivery</td>
<td>8</td>
<td>BB BGN4 + BL AD011 + LA AD031 malt dextrin and alpha-corn without probiotic bacteria</td>
<td>Sachets</td>
<td>$1.6 \times 10^{9}$ CFU/day for each probiotic strain, daily</td>
<td></td>
</tr>
<tr>
<td>(Dotterud, et al., 2010; Simpson, et al., 2015)</td>
<td>Yes</td>
<td>Prenatally and postnatally in mothers</td>
<td>From 36 wks</td>
<td>36wks to 3mon Postnatal</td>
<td>3.5-4</td>
<td>LGG + BA (subsp. lactis Bb-12) + La-5 Sterile milk (with no probiotic bacteria)</td>
<td>Milk</td>
<td>$5 \times 10^{10} + 5 \times 10^{9}$ CFU/day, 250mL daily</td>
<td></td>
</tr>
<tr>
<td>Primary article</td>
<td>Comparable baseline characteristic</td>
<td>Participants receiving intervention</td>
<td>Timing of Intervention in pregnancy</td>
<td>Intake of Intervention from/until</td>
<td>Duration of intervention (months)**</td>
<td>Probiotic organism***</td>
<td>Placebo</td>
<td>Mode of delivery</td>
<td>Total daily dose (Colony Forming Units)</td>
</tr>
<tr>
<td>-----------------</td>
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<td>------------------------------------</td>
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<td>--------------------------------------</td>
</tr>
<tr>
<td>(Boyle et al., 2011)</td>
<td>Yes</td>
<td>Prenatally</td>
<td>From 36 wks.</td>
<td>36wks to delivery</td>
<td>2-4 wks.</td>
<td>LGG</td>
<td>malt dextrin</td>
<td>Capsule</td>
<td>1 × 10^{10} CFU/day, daily</td>
</tr>
<tr>
<td>(Rautava, et al., 2012)</td>
<td>Yes</td>
<td>Prenatally and postnatally in mothers</td>
<td>2mon before expected delivery day</td>
<td>28wks to 2mon postnatal</td>
<td>4</td>
<td>LPR+BL999 or ST11+BL999</td>
<td>Same dietary supplement without probiotics</td>
<td>Sachets</td>
<td>1 × 10^{9} CFU/day, daily for each probiotic strain</td>
</tr>
<tr>
<td>(Ou et al., 2012)</td>
<td>Yes</td>
<td>Prenatally and postnatally in mothers and infants</td>
<td>Beginning from 24wks</td>
<td>24wks to 6mon postnatal</td>
<td>9.5-10</td>
<td>LGG</td>
<td>Micro-crystalline cellulose</td>
<td>Capsule</td>
<td>1 × 10^{10} CFU/day, daily</td>
</tr>
<tr>
<td>(Abrahamsson, et al., 2013)</td>
<td>Yes, higher Antibiotic prescription during the first year of life in Int. vs. placebo in 1'sy of life</td>
<td>Prenatally and postnatally in infants</td>
<td>From 36+0 wks.</td>
<td>36wks to 12mon postnatal</td>
<td>12.5-13</td>
<td>L. reuteri</td>
<td>Same oil without any bacteria</td>
<td>Oil drops</td>
<td>1 × 10^{8} CFU/day, 5 drops daily</td>
</tr>
<tr>
<td>(Wickens et al., 2013)</td>
<td>Yes</td>
<td>Prenatally and postnatally in mothers and infants</td>
<td>From 35wks gestation</td>
<td>35wks to 2yrs. postnatal</td>
<td>25-25.5</td>
<td>BA HN019 or L rhamnosus HN001</td>
<td>Dextran, salt, and a yeast extract</td>
<td>Capsule</td>
<td>9 × 10^{9}, 6 × 10^{9} CFU/day, daily</td>
</tr>
<tr>
<td>(Allen et al., 2014)</td>
<td>Yes</td>
<td>Prenatally and postnatally in infants</td>
<td>From 36 wks.</td>
<td>36wks to 6mon postnatal</td>
<td>7</td>
<td>LS + LP + BB + BA</td>
<td>malt dextrin powder</td>
<td>Vegetarian capsule</td>
<td>1 × 10^{10} CFU/day, daily</td>
</tr>
</tbody>
</table>

*Abbreviations: WKS= weeks, MON= months, BF=Breast-Feeding
**Indicates total duration in pregnancy plus after birth either in mothers only or both mother and infant, if applicable
***LS=Lactobacillus Salivarius , LP=Lactobacillus Paracasei, LGG=Lactobacillus rhamnosus GG, La-5=Lactobacillus.acidophilus 5, LL=Lactococcus. Lactis, LA=Lactobacillus.acidophilus, BA=Bifidobacterium Animalis, BB=Bifidobacterium Bifidum, BL= Bifidobacterium Lactis, Bb=Bifidobacterium breve, Pf=Propionibacterium freudenreichii
3.5.4. Risk of bias in studies of maternal pro/prebiotic consumption during pregnancy and prevention of allergic diseases in the offspring

The summary of risk of bias in trials on pro/prebiotic studies is presented in Figure 3.2. The reviewer’s judgment for the risk of bias assessment of pro/prebiotic studies is shown in appendix 3.6.
<table>
<thead>
<tr>
<th>Study author</th>
<th>Random Sequence Generation</th>
<th>Allocation Concealment</th>
<th>Double Blinding</th>
<th>Blinding of Outcome Assessment</th>
<th>Incomplete Outcome Data</th>
<th>Selective Outcome Reporting</th>
<th>Other Sources of Bias</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kalliomäki (2003)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>?</td>
</tr>
<tr>
<td>Kalliomaki (2007)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>?</td>
</tr>
<tr>
<td>Huurre (2008)</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Kopp (2008)</td>
<td>+</td>
<td>?</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Kuitunen (2009)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>?</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Kim (2010)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Dotterud (2010)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>?</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Boyle (2011)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ou (2012)</td>
<td>?</td>
<td>+</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>?</td>
</tr>
<tr>
<td>Rautava (2012)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Abrahamsson (2013)</td>
<td>?</td>
<td>+</td>
<td>?</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Wickens (2013)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Allen (2014)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Gorissen (2014)</td>
<td>?</td>
<td>?</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>?</td>
</tr>
<tr>
<td>Simpson (2015)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Random Sequence Generation: 69% Green, 31% Yellow
Allocation Concealment: 75% Green, 25% Red
Double Blinding: 50% Green, 25% Red
Blinding of Outcome Assessment: 75% Green, 6% Red
Incomplete Outcome Data: 44% Green, 18% Red
Figure 3.2. Summary of risk of bias assessment in the included trials of pro/prebiotics and prevention of allergy in the offspring
3.5.4.1. Random sequence generation (selection bias)

Of the 16 included trials, eleven studies were assessed as having a low likelihood of selection bias (Allen et al., 2014; Boyl et al., 2011; Dotterud et al., 2010; Kalliomaki et al., 2003; Kalliomäki et al. 2007; Kim et al., 2010; Kopp et al., 2008; Kuitunen et al., 2009; Rautava et al., 2012; Simpson et al., 2015; Wickens et al., 2013). The treatment allocation in these studies was carried out using a computer generated randomisation system. The risk of selection bias in the remaining studies was unclear as they either had not reported their method of randomisation or just stated that they had used block randomisation with no further information.

3.5.4.2. Allocation concealment

The method of allocation concealment was not clear in four studies (Gorissen et al., 2014; Huurre, et al., 2008; Kopp et al., 2008; Niers et al., 2009). The remaining studies kept the allocation concealed from the staff involved with the study.

3.5.4.3. Double blinding (performance bias)

There was no blinding of either staff and/or participants at the time of extended follow-up in four studies (Abrahamsson, et al., 2013; Gorissen et al., 2014; Simpson et al., 2015; Wickens et al., 2013). Four studies gave no indication of blinding, of either staff or participants, and were recorded as having an unclear risk (Huurre et al., 2008; Kalliomaki et al., 2007; Kopp et al., 2008; Niers et al., 2009). The rest of the studies stated that their trial was double-blinded by keeping the codes blinded to research staff throughout the study and also, by ensuring an equal appearance and smell for the intervention and placebo supplementations.

3.5.4.4. Blinding of outcome assessment (detection bias)

Only three of the 16 included studies were rated as having high risk of bias for blinding of outcome assessment (Abrahamsson, et al., 2013; Gorissen et al., 2014; Simpson et al., 2015), as their extended follow-up study was only single-blinded to either the parents and/or investigator(s). One study was rated as unclear for the blinding of outcome assessment (Kuitunen, et al., 2009) and the remainder were classified as low risk.
3.5.4.5. Incomplete outcome data (attrition bias)
Completeness of data was ranked as high risk in six studies (Abrahamsson et al., 2013; Huurre et al., 2008; Kalliomaki et al., 2007; Kim et al., 2010; Simpson et al., 2015; Wickens et al., 2013). Two studies had a high loss to follow-up and did not specify the reasons for missing participants (Abrahamsson et al., 2013; Kalliomäki et al., 2007) and one reported the outcomes for different sub-samples within study arms (Huurre et al., 2008). Wickens et al. (2013) used imputed analysis for a number of the reported outcomes and the two remaining studies (Kim et al., 2010 and Simpson et al., 2015) were affected by a high rate of attrition due to participants’ refusal and non-compliance. Three studies (Dotterud et al., 2010; Kuitunen, et al., 2009; Ou et al., 2012) were rated as unclear as they did not specify the reasons for loss to follow-up. The rest of the studies were rated as low risk of bias as missing data were balanced across groups.

3.5.4.6. Selective outcome reporting (reporting bias)
All the pre-specified outcomes, either published in the protocol for the study or listed in the manuscript, were reported including the cumulative prevalence of allergic disorders and consequently all the included trials were deemed to have a low risk of bias.

3.5.4.7. Other potential sources of bias
Each included study was assessed for other factors that might contribute to additional risk of bias. Two studies were rated as being at high risk of further bias (Abrahamsson et al., 2013; Huurre, et al., 2008). In one, participants reported that they continued to consume the study intervention product after delivery despite this not being part of the study protocol (Abrahamsson et al., 2013). In the other, Huurre et al. (2008) reported no information as to whether women have been aware of their allocation or whether they have consumed the probiotic strain(s) after their intervention was completed. Four studies were rated as unclear for further risk of bias (Gorissen et al., 2014; Kalliomaki et al., 2003; Kalliomäki et al., 2007, Ou et al., 2012) since there was no information provided regarding whether the participants have consumed the pro/prebiotics supplement afterwards. The remaining studies were classified as low risk of any further bias.
3.5.5. Meta-analyses of effectiveness of maternal pro/prebiotics consumption during pregnancy and prevention of allergic diseases in the offspring

Pooled results from meta-analyses in the studies that examined the effectiveness of maternal pro/prebiotic supplementations during pregnancy for the prevention of allergic outcomes in offspring are presented in the following section. Funnel plots are presented for outcomes that have been reported by a sufficient number of studies (≥10).

3.5.5.1. Any ‘Allergic Diseases’ as an outcome for pro/prebiotic intervention

The use of pro/prebiotic products during pregnancy for prevention of allergic diseases was assessed in three studies. Figure 3.3 shows the Forest plot for pro/prebiotics versus placebo in pregnant women for the prevention of allergic diseases in offspring. Any ‘allergic disease’ was defined differently in the included studies. These are described as below:

**Abrahamsson et al. (2013):** Allergic disease was defined as “asthma, allergic rhinoconjunctivitis (ARC), allergic urticaria and eczema” with the child having had symptoms of and/or having been treated for the actual allergic disease during the last 12 months. Children with allergic disease before school age who did not have any symptoms during the last 12 months were defined as healthy. This outcome was measured at 2 and 7 year follow-ups and reported as positive at any of the time points.

**Kuitunnen et al. (2009):** The outcome considered was ‘allergic diseases with or without positive SPT response and/or IgE >0.7kU/L’. Allergic diseases were defined as food allergy, eczema, asthma or allergic rhinitis. The outcome was measured as a cumulative prevalence at 5 years.

**Ou et al. (2012):** Any allergic disease was defined by any phenotypes of allergy as physician-diagnosed asthma, allergic rhinitis, and atopic dermatitis (AD), plus sensitisation. The outcome was reported as point prevalence at 3 years.

Statistically, these studies were largely homogeneous, with 0% variation between studies attributable to heterogeneity as opposed to sampling error (χ²=0.49, p=0.78, I²=0%). The result of meta-analysis did not show an association between maternal intake of pro/prebiotics during pregnancy and prevention of allergic diseases in the
offspring (Risk Ratio (RR)= 0.979, 95% Confidence Interval (CI)= 0.88-1.086; 1,157 children) (Figure 3.3).

**Figure 3.3. Forest plot of pro/prebiotics vs. placebo for any allergic diseases**

Measure: Binary: risk ratio  
Heterogeneity: Q = 0.497; df = 2; p = 0.78; I-squared = 0%; tau-squared = 0.  
Random effects model: 0.979 (0.881, 1.09)

<table>
<thead>
<tr>
<th>Outcome: Any allergic diseases</th>
<th>Probiotics n/N</th>
<th>Placebo n/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abrahamsson</td>
<td>56/94</td>
<td>51/90</td>
</tr>
<tr>
<td>Kuitunne</td>
<td>234/445</td>
<td>245/446</td>
</tr>
<tr>
<td>Ou</td>
<td>11/41</td>
<td>12/41</td>
</tr>
<tr>
<td><strong>Subtotal</strong></td>
<td><strong>580</strong></td>
<td><strong>577</strong></td>
</tr>
</tbody>
</table>

3.5.5.2. Asthma as an outcome measure for pro/prebiotic intervention

Seven included studies measured the effect of pro/prebiotic products consumption during pregnancy on prevention of asthma in offspring (Figure 3.4). In studies included in the meta-analysis asthma was defined as follows:

**Abrahamsson et al. (2013):** Two criteria were used for the diagnosis of Asthma: 1) doctor diagnosis and asthma symptoms and/or use of asthma medication during the last 12 months; 2) wheeze or nocturnal cough and a positive reversibility test and/or pathological fractional exhaled nitric oxide (FE\textsubscript{NO}) value. This outcome was measured at 2 and 7 years follow-ups and reported as positive at any of the time points.

**Allen et al. (2014):** All reported asthma was used whether diagnosed by a health professional or not, and assessed by follow-up questionnaires. This outcome was reported as the cumulative prevalence at 2 years.

**Gorrisen et al. (2014):** Asthma was defined based on at least one of the following four criteria: i) doctors diagnosed asthma active in the past 12 months; ii) parental reported wheezing in the past 12 months; iii) use of asthma medication in the past 12 months; iv) an at least 9% reversibility in the forced expiratory volume in half a second Forced Expiratory Volume (FEV\textsubscript{0.5}) or in one second (FEV1). The outcome
was reported as point prevalence at 6 years

**Kalliomaki et al. (2007):** Asthma was defined by whether the child had been qualified by the Social Insurance Institution of Finland for a special reimbursement for asthma medication. This outcome was reported as the point prevalence at 7 years.

**Kuitunnen et al. (2009):** The outcome considered was ‘Asthma all, with or without sensitisation’. It was diagnosed if the child had 2 doctor-diagnosed wheezing episodes plus continuous cough, exercise-induced symptoms or verified reversible bronchial obstruction in oscillometry. The outcome was reported as the cumulative prevalence at 5 years.

**Simpson et al. (2015):** The outcome considered was ‘Current Asthma’ measured at 6 years. This was defined by a positive answer to both questions “Has your child ever been diagnosed with asthma by a doctor?” and “In the past 12 months, has your child been treated with tablets, inhalers or other medications for wheezing, chest tightness or asthma?”.

**Wickenze et al. (2013):** Current Asthma was diagnosed using ISAAC questionnaire for a history of asthma plus wheeze or inhaler use in the last 12 months in children. This outcome was reported as the point prevalence at 6 years.

The results of the pooled analysis for asthma risk reduction in offspring following pro/prebiotic intervention during pregnancy are shown in Figure 3.4. There was no evidence of statistical heterogeneity between studies ($\chi^2=5.77, P=0.44, I^2=0\%$). The result of meta-analysis did not show an association between maternal intake of pro/prebiotics during pregnancy and prevention of asthma in the offspring (RR=1.04, 95% CI= 0.85-1.27, 2,317 children).

**Figure 3.4. Forest plot of pro/prebiotics vs. placebo for asthma**

Measure: Binary: risk ratio  
Heterogeneity: $Q = 5.77; df = 6; p = 0.449; I$-squared = 0%; $\tau$-squared = 0.  
Random effects model: 1.04 (0.851, 1.27)
3.5.5.3. Eczema as an outcome measure for pro/prebiotic intervention

The meta-analysis on the maternal consumption of pro/prebiotics during pregnancy and its effectiveness on prevention of eczema in the offspring is shown in Figure 3.5. In total, 13 studies were included in the meta-analysis. Included trials used different definitions of eczema but for this systematic review, all the definitions were considered. The definition of eczema used by trials included in the meta-analysis were as below:

**Abrahamsson et al. (2013):** The outcome considered was ‘Eczema, IgE-associated or not’. Eczema was defined as a pruritic, chronic or chronically relapsing non-infectious dermatitis with typical features and distribution, as suggested by Hanifin (Hanifin & Rajka 1980). This outcome was measured at 2 and 7 years follow-ups and reported as positive at any of the time points.

**Allen et al. (2014):** All reported eczema of any duration and whether diagnosed by a health professional or not were used in this study. Eczema was defined as an itchy rash affecting the face, scalp or extensor surfaces of the limbs in infants and flexures in older children and of duration ≥4 weeks and with ≥1 exacerbation by age 24 months based on the information from follow-up questionnaires. This outcome was measured as the cumulative prevalence at 2 years.

**Boyle et al. (2011):** Eczema ever was defined according to the UK Eczema Working
Party criteria (Williams, Burney, Pembroke, & Hay 1994). The definition included a history of itchy skin, scratching or rubbing plus at least three of the following: family history of atopic disease; history of generally dry skin; history of skin rash affecting the flexures, cheeks or outer surfaces of the limbs; onset of rash under the age of 2 years; visible dermatitis at any study visit affecting the flexures, cheeks or outer surfaces of the limbs. The outcome for this study was reported as the point prevalence at 1 year.

Huurre et al. (2008): Atopic eczema was diagnosed using the Hanifin criteria. The outcome was reported as the point prevalence at 1 year.

Kalliomaki et al. (2007): The diagnosis of eczema was made on the basis of both a questionnaire and a clinical examination. In particular, diagnosis of eczema was confirmed if there had been pruritic eczematous lesions with typical location and with relapsing or chronic course during the last 12 months. This study reported the outcome as the cumulative risk for developing eczema during the first 7 years of life.

Kim et al. (2010): Eczema was confirmed when the skin lesions met the criteria of Hanifin. This study reported the outcome as the cumulative incidence at 1 year.

Kopp et al. (2008): The outcome considered was ‘atopic dermatitis’ and was confirmed by pruritus, facial or extensor involvement, or both, and chronic relapsing course by using Williams UK Working Party’s criteria (Williams, et al., 1994). Physicians who were blinded to the allocated treatment conducted the physical examinations. The outcome was reported as the cumulative incidence during the first 2 years.

Kuitunen et al. (2009): The outcome considered was ‘all eczema, IgE and non-IgE mediated’. Eczema was diagnosed according to the Williams UK Working Party’s criteria (Williams, et al., 1994) which meant an itchy skin plus 3 or more of the following: family history of atopic disease, dry skin during the previous 12 months, history of eczema, or visible eczema at typical sites. The outcome was reported as the cumulative prevalence at 0-5 years.

Gorissen et al. (2014): Eczema was defined according to the Williams UK Working Party’s criteria (Williams, et al., 1994). This outcome was reported as the point prevalence at 6 years.

Ou et al. (2012): The outcome considered was ‘Eczema ever’ and assessed using the modified ISAAC questionnaire including the physician-diagnosis of atopic dermatitis. This outcome was reported as the point prevalence at 3 years.
Rautava et al. (2012): Eczema was diagnosed according to the criteria introduced by Hanifin (Hanifin & Rajka, 1980), based on the following features: pruritus, typical morphology and distribution, and a chronic relapsing course. The last criterion was fulfilled if the infant had 2 episodes of eczema with duration of at least 1 month each during the first 2 years of life. If the skin condition persisted without periods of remission, the eczema was considered chronically persistent. This outcome was reported as the cumulative incidence at 2 years.

Simpson et al. (2015): The outcome considered was ‘atopic dermatitis’ and was assessed during the clinical examination(s) using the Williams UK Working Party’s criteria (Williams, et al., 1994) and were asked about having atopic dermatitis at any point up to 6 years. This study reported the outcome as the cumulative incidence estimate at 6 years.

Wickens et al. (2013): Eczema was assessed by study nurses at follow-up and determined as present if there was a history of an itchy rash since turning 4 years, plus 2 or more of the following: (i) a generally dry skin since 4 years, (ii) a history of asthma or hay fever ever, (iii) flexural involvement since 4 years around the eyes, sides or fronts of the neck, elbow or knee flexures, or fronts of ankles, (iv) visible atopic eczema present at any of these sites. This outcome was measured at 2, 4 and 6 years follow-ups and reported as positive at any of the time points.

For Gorrisen et al. (2014), the cumulative prevalence of atopic eczema was reported in the earlier report of this study (Niers et al., 2009) and their results showed no difference in the cumulative incidence of atopic eczema during the 2 years follow-up period ($\chi^2$ test, p=0.876). However, the cumulative incidence of parental reported eczema at 1 year [23/50 (probiotics) vs. 30/48 (placebo)] and 2 years [27/50 (probiotics) vs. 33/48 (placebo)] showed a significant difference between the groups (p<0.05).

A high level of statistical heterogeneity was observed between studies ($\chi^2=35.5$, P=0.00039, $I^2=66.2\%$). The results of meta-analysis showed an association between maternal intake of pro/prebiotics during pregnancy and prevention of eczema in the offspring (RR=0.79, 95% CI=0.67-0.93, 3,271 children).

Figure 3.5. Forest plot of pro/prebiotics vs. placebo for any eczema

Measure: Binary: risk ratio
Heterogeneity: $Q = 35.3$; $df = 12$; $p = 0.000422$; $I^2$-squared = $66\%$; $\tau^2$-squared = $0.0468$.

Random effects model: $0.791 \ (0.674, \ 0.928)$

Visual inspection of the funnel plot for trials on pro/prebiotics for prevention of any eczema in children suggests no likelihood of publication bias. Although some evidence of variances between studies in terms of sample size and methodological quality is evident.
3.5.5.4. Wheeze as an outcome measure for pro/prebiotic intervention

In total seven studies were included in a meta-analysis measuring the effect of pro/prebiotic intake during pregnancy for prevention of wheeze in the offspring (Figure 3.7). The definitions of wheeze, as described in the included trials, were as follows:

Abrahamsson et al. (2013): Wheeze was defined as an episode with obstructive airway symptoms. This outcome was measured at 2 and 7 years follow-ups and reported as positive at any of the time points.

Allen et al. (2014): Wheezing without symptoms of a virus infection (assessed by follow-up questionnaires). It is not clear whether the outcome reported as cumulative or point prevalence at 2 years.

Boyle et al. (2011): History of wheeze (undefined) was recorded as the outcome. This outcome was reported as the point prevalence at 1 year.

Kopp et al., 2008: Wheeze was defined as $\geq 5$ episodes of wheezing bronchitis during the first 2 years of life

Ou et al. (2012): Wheeze-ever was defined using the modified ISAAC questionnaire asking about symptoms of wheezing. This outcome was reported as the point prevalence at 3 years.
**Simpson et al. (2015):** Wheeze was defined by a positive answer to both questions “Has your child ever had whistling in the chest?” and “Has your child ever had episodes of wheezing or tightness in the chest?”. This outcome was reported as cumulative incidence at 6 years.

**Wickens et al. (2013):** Wheeze was defined by asking standard questions relating to wheezing from the ISAAC questionnaire. This outcome was reported for cases completing at least one follow-up time point at 2, 4 and 6 years.

A moderate level of heterogeneity was observed between studies ($\chi^2=11.3$, $P=0.07$, $I^2=46.8\%$). The result of meta-analysis did not show an association between maternal intake of pro/prebiotics during pregnancy and prevention of wheeze in the offspring (RR=0.91, 95% CI= 0.73-1.36, 1,773 children).

**Figure 3.7. Forest plot of pro/prebiotics vs. placebo for wheeze**

Measure: Binary: risk ratio
Heterogeneity: $Q = 11.3$; df = 6; $p = 0.0799$; I-squared = 46.8%; tau-squared = 0.0352.
Random effects model: 0.913 (0.733, 1.14)

<table>
<thead>
<tr>
<th>Outcome: Wheeze</th>
<th>Probiotics n/N</th>
<th>Placebo n/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abrahamsson</td>
<td>18/94</td>
<td>14/90</td>
</tr>
<tr>
<td>Allen</td>
<td>50/214</td>
<td>55/171</td>
</tr>
<tr>
<td>Boyle</td>
<td>27/122</td>
<td>29/120</td>
</tr>
<tr>
<td>Kopp</td>
<td>13/50</td>
<td>4/44</td>
</tr>
<tr>
<td>Ou</td>
<td>14/65</td>
<td>11/64</td>
</tr>
<tr>
<td>Simpson</td>
<td>46/132</td>
<td>55/142</td>
</tr>
<tr>
<td>Wickens</td>
<td>189/309</td>
<td>102/156</td>
</tr>
<tr>
<td><strong>Subtotal</strong></td>
<td>986</td>
<td>787</td>
</tr>
</tbody>
</table>
3.5.5.5. Rhinitis as an outcome measure for pro/prebiotic intervention

Four studies were included in a meta-analysis measuring the effect of pro/prebiotic intake during pregnancy for prevention of rhinitis in the offspring (Figure 3.8). For the purpose of this systematic review, only studies that have reported “Rhinitis” as an outcome measure are considered and studies that reported “Allergic Rhinoconjunctivitis” as an allergic outcome were excluded (reported by: Abrahamsson et al., 2013; Wickens et al., 2013; Simpson et al., 2015). The definitions of rhinitis in the included trials were as follows:

**Allen et al. (2014):** Allergic rhinitis (reported or diagnosed by a health professional). This outcome was reported as the cumulative prevalence at 2 years.

**Gorissen et al. (2014):** Allergic rhinitis was defined according to the Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines. This outcome was reported as the point prevalence at 6 years.

**Kalliomaki et al. (2007):** Allergic rhinitis was defined as nasal discharge, blockage, sneezing, and itching related to allergen exposure and sensitisation. This outcome was reported as the point prevalence at 7 years.

**Kuitunen et al. (2009):** The outcome considered was ‘rhinitis, positive inhalant SPT response, sIgE level >0.7 kU/L, or both’. Rhinitis was defined according to ARIA guidelines as 2 or more symptoms of nasal discharge, blockage, and sneeze/itch recurrently during antigen contact. This outcome was reported as cumulative prevalence at 0-5 years.

There was a moderate level of statistical heterogeneity between studies ($\chi^2=4.37, \ P=0.19, \ I^2=36.6\%$). The results of meta-analysis did not show an association between maternal intake of pro/prebiotics during pregnancy and prevention of rhinitis in the offspring (RR=1.36, 95% CI=0.84-2.19, 1,479 children) (Figure 3.8).

**Figure 3.8. Forest plot of pro/prebiotics vs. placebo for rhinitis**

Measure: Binary: risk ratio
Heterogeneity: $Q = 4.73; \ df = 3; \ p = 0.192; \ I\text{-squared} = 36.6\%; \ tau\text{-squared} = 0.0909.$
Random effects model: 1.36 (0.842, 2.19)
3.5.5.6. Food allergy as an outcome measure for pro/prebiotic intervention

In total three studies were included in a meta-analysis measuring the effect of pro/prebiotic intake during pregnancy for prevention of food allergy in the offspring (Figure 3.9). Food allergy was defined as below:

**Allen et al. (2014):** Any reported food allergy (undefined). This outcome was reported as the point prevalence at 2 years.

**Gorissen et al. (2014):** Food allergy was defined as doctor diagnosed food allergy, combined with sensitisation to food allergens as well as assessment using a slightly modified ISAAC questionnaire. This outcome was reported as the point prevalence at 6 years.

**Kalliomaki et al. (2003):** Allergies to cow’s milk was diagnosed by DBPCC and was reported as the point prevalence at 4 years.

Studies were largely homogeneous with no variation observed between the included trials ($\chi^2=2.04$, $P=0.56$, $I^2=0\%$). The result of meta-analysis did not show an association between maternal intake of pro/prebiotics during pregnancy and prevention of food allergy in the offspring (RR=0.85, 95% CI=0.54-1.32, 594 children) (Figure 3.9).

**Figure 3.9. Forest plot of pro/prebiotics vs. placebo for food allergy**

Measure: Binary: risk ratio  
Heterogeneity: $Q=2.04$; df = 3; $p = 0.564$; I-squared = 0%; tau-squared = 0.  
Random effects model: 0.812 (0.506, 1.3)
3.5.5.7. Raised specific Immunoglobulin E (sIgE) as an outcome measure for pro/prebiotic intervention

Six studies were included in a meta-analysis examining the effect of pro/prebiotic intake during pregnancy and its possible effect on a positive sIgE result in offspring (Figure 3.10). The definition of a positive sIgE result described by different studies is reported below:

**Dotterud et al. (2010):** A positive level of sIgE was defined as sIgE ≥0.35 kU/L. sIgE was measured for mite (Dermatophagoides pteronyssinus), mould (Cladosporium herbarum), cat and dog dander, birch, timothy (grass) and mugwort pollen, hen’s egg white, codfish, hazelnut and peanut allergens. This outcome was reported as the point prevalence at 2 years.

**Kim et al. (2010):** sIgE to food was measured against common food allergens (egg white, cow’s milk, wheat, peanut, soybean, and buckwheat). Antigen-specific IgE levels ≥0.35 kU/L were considered positive. This outcome was reported as the point prevalence at 1 year.

**Kuitunne et al. (2009):** Positive level of any sIgE was defined as sIgE >0.7 kU/L. sIgE antibodies were measured against milk, egg white, birch, timothy, cat and dog, peanut, and D pteronyssinus by using the ImmunoCAP system. This outcome was reported as the cumulative prevalence at 0-5 years.

**Niers et al. (2009):** Positive result was defined as a concentration of sIgE ≥0.35 IU/ml. sIgE was conducted for food panel of egg white, cow’s milk, peanut, house dust mite, and cat dander epithelium on the IMMULITE 2000. This outcome was reported as the point prevalence at 2 years.

**Ou et al. (2012):** The outcome considered was ‘sensitisation by the detection of one
or more common allergen-specific IgE antibodies in blood’. Positive level of sIgE was defined as sIgE >0.7 kU/L. Allergen-sIgE sensitisation was assessed to six common allergens, two common inhaled allergens [Dermatophagoides pteronyssinus (Der p) and cockroach], and four common food allergens (egg white, milk protein, shrimp, and peanut), which have prevalence of sensitisation greater than 5% in the study country, Taiwan. This outcome was reported as the point prevalence at 3 years.

Wickens et al. (2013): Positive sIgE was defined as sIgE ≥0.35 kU/L. sIgE was measured to six allergens (egg white, peanut, cow’s milk, cat pelt, Dermatophagoides pteronyssinus, and mixed grass pollen) using Stallergenes 1 mm lancets. This outcome was reported as the point prevalence at 6 years.

It is important to note that the cut-off to determine a positive sIgE result differed across the studies and, with the exception of the study by Dotterud et al (2010); all studies were conducted in high-risk population with a family history of atopy. A moderate level of heterogeneity was observed between the studies ($\chi^2=6.26$, $P=0.28$, $I^2=20.1\%$). The results of meta-analysis (Figure 3.10) did not show an association between maternal intake of pro/prebiotics during pregnancy and positive sIgE in children (RR=1.02, 95% CI=0.86-1.20, 1,543 children).

Also the meta-analysis including only studies with a cut-off of ≥0.35 kU/L to measure a positive sIgE did not modify the results (RR=1.08, 95% CI=0.774, 1.49) (Forest plot not shown).

Figure 3.10. Forest plot of pro/prebiotics vs. placebo for positive sIgE in offspring

Measure: Binary: risk ratio
Heterogeneity: $Q = 6.26; df = 5; p = 0.282$; $I$-squared = 20.1%; tau-squared = 0.00861
Random effects model: 1.02 (0.864, 1.2)
### 3.5.5.8. Positive Skin Prick Test (SPT) to any allergen as an outcome measure for pro/prebiotic intervention

Ten studies were included in a meta-analysis measuring the effect of pro/prebiotic intake during pregnancy and sensitisation, as measured by SPT in the offspring (Figure 3.11). Sensitisation was measured by SPT and a positive reaction was described in the included trials as below:

**Abrahamsson et al. (2013):** The outcome considered was ‘any positive SPT’. SPTs were carried out with egg white, fresh skimmed cow’s milk (lipid concentration 0.5%) and standardised cat, dog, birch, peanut, mite (Der p) and timothy extracts. Histamine hydrochloride (10 mg/ml) was used as positive and albumin diluent as negative control. The test was regarded as positive if the mean diameter of the wheal was ≥3 mm. This outcome was measured at 2 and 7 years follow-ups and reported as positive at any of the time points.

**Allen et al. (2014):** The outcome considered was ‘any positive SPT’. The tests were performed using common food allergens (cow’s milk, hen’s egg), aeroallergens (house dust mite, cat dander, grass pollen) and positive (histamine) and negative controls. The response to an allergen was considered positive if there was a wheal diameter ≥3 mm. This outcome was reported as the point prevalence at either 6 months or 2 years.
Boyle et al. (2011): The outcome considered was ‘any positive SPT’. The tests were performed to house dust mite, cat, ryegrass pollen, cow’s milk, egg and peanut positive (10% histamine chloride) and negative (glycerin-saline) controls. Atopy was defined as a SPT wheal diameter ≥3 mm greater than the negative control to any single allergen tested. This outcome was reported as the point prevalence at 1 year.

Dotterude et al. (2010): The outcome considered was ‘any positive SPT’. The tests were measured to mite (*Dermatophagoides pteronyssinus* = Der *p*), mould (*Cladosporium herbarum*), cat and dog dander, birch, timothy (grass) and mugwort pollen, hen’s egg white, codfish, hazelnut and peanut allergens. For cow’s milk, fresh skimmed milk was used. It is not clear what wheal diameter was considered as a positive result; the paper only reports that the reading of the tests followed standardised procedures. This outcome was reported as the point prevalence at 2 years.

Huurre et al. (2008): The outcome considered was ‘Positive SPT’. The antigens tested for SPT included cow’s milk, egg white, wheat and rice flour both diluted 1/10 (w/v) with 0.9% (w/v) sodium chloride, gliadin diluted 1 mg/mL with an ethanol/glyceroleum/ALK-diluent mixture, cod, soya bean, birch, six grasses, cat, dog, Der *p* allergen, latex and potato, carrot and banana by prick–prick technique. There is no information what wheal diameter was considered as a positive result. This outcome was reported as the point prevalence at 2 years.

Kalliomaki et al. (2003): The outcome considered was ‘any positive SPT’. The tests were conducted to cow’s milk, egg white, wheat flour diluted 1/10 (wt/vol) with 0.9% (wt/vol) sodium chloride, gliadin diluted 1/1000 (wt/vol) with 0.9% (wt/vol) sodium chloride, cod, soya bean, hazelnut, peanut, birch, mugwort, alder, 6 local grasses, cat, dog, and Der *p* allergen, and latex. A test was considered positive if a wheal of ≥3 mm was observed in response to any of the allergens in the presence of an appropriate response to the positive control (10 mg/mL histamine dihydrochloride) and no response to the negative control (allergen diluent; ALK-Abello). This outcome was reported as the point prevalence at 4 years.

Kuitunen et al. (2009): The outcome considered was ‘Any positive SPT’. The tests were performed to cat, dog, birch, timothy, mugwort, Der *p*, cow’s milk, egg, wheat, and peanut allergens at 2 and 5 years with commercial solutions or fresh food dilutions with 0.9% sodium chloride. Positive and negative controls were as histamine chloride and glycerin respectively. A wheal diameter of ≥3 mm in the presence of
negative control was considered positive according to the European Academy of Allergology and Clinical Immunology (EAACI) recommendations. This outcome was reported as the cumulative prevalence at 0-5 years.

**Niers et al. (2009):** The outcome considered was ‘Positive SPT’. The tests were performed with allergen extracts for egg white, cow’s milk, peanut, hazelnut, cat, dog, house dust mite (*Der p*), birch, and grass. A wheal diameter of ≥3 mm at 15 min was considered positive. This outcome was reported as the point prevalence at 2 years.

**Rautava et al. (2012):** The outcome considered was ‘SPT positive’. The tests were performed for cow’s milk, egg white, and wheat and rice flour both diluted 1/10 (w/v) with 0.9% (w/v) sodium chloride, gliadin diluted 1 mg/mL with an ethanol/glyceroleum/ ALK-diluent mixture, cod, soy bean, birch, 6 grasses, cat, dog, *Der p* allergen, latex, potato, carrot, and banana by prick-prick technique. Reactions were read at 10 to 15 minutes and a mean diameter of the wheal of at least 3 mm was considered in the presence of a negative control. This outcome was reported as the point prevalence at 2 years.

**Wickens et al. (2013):** The outcome considered was ‘SPT sensitisation’. Australian Society of Clinical Immunology and Allergy (ASCIA) guidelines were used to measure SPT reactions to egg white, peanut, cow’s milk, cat pelt, *Der p*, and mixed grass pollen. SPT sensitisation was defined as a mean wheal diameter ≥3 mm to one or more allergens after subtraction of the negative control. This outcome was reported as the cumulative prevalence at 6 years.

Statistically, these studies were largely homogeneous with no variation between studies ($\chi^2$=6.56, $P=0.68$, $\Gamma^2=0\%$). The result of meta-analysis did not show an association between maternal intake of pro/prebiotics during pregnancy and prevention of sensitisation in the offspring (RR=0.91, 95% CI=0.82-1.02, 2,877 children).

**Figure 3.11. Forest plot of pro/prebiotics vs. placebo for sensitisation to any allergen, measured by SPT**

Measure: Binary: risk ratio
Heterogeneity: $Q = 6.56; df = 9; p = 0.683; I^2$-squared = 0%; tau-squared = 0.
Random effects model: 0.917 (0.822, 1.02)
Visual inspection of the funnel plot for trials on pro/prebiotics for prevention of childhood sensitisation suggests the possibility of publication bias e.g. statistical power of studies, varied methodological quality.
3.5.6. Discussion of the evidence synthesis of pro/prebiotics consumption during pregnancy and prevention of allergic diseases in the offspring

3.5.6.1. Summary of main results

This systematic review summarised data from 16 placebo-controlled randomised controlled trials (PC-RCTs) of maternal intake of pro/prebiotics for prevention of childhood allergies, including a total of 3,567 children with follow-up duration ranging from 1-7 years (mean 3.56 years). Trials were at risk of bias with 25% rated either unclear or high risk for their performance bias. Studies also had varied duration of follow-up and generally had small sample sizes. Wide variety was observed for the choice of probiotic strain and there were little similarity between studies for dosage, timing and duration of intervention. The findings from this systematic review and meta-analysis do not provide evidence of a protective association between intake of pro/prebiotics during pregnancy and subsequent development for a number of allergic conditions including asthma, wheeze, rhinitis, food allergy and sensitisation to allergens as measured by sIgE and SPTs. However, there appeared to be an effect for eczema. The meta-analysis of thirteen studies showed a significantly reduced risk of developing any eczema in offspring (RR=0.79, 95% CI=0.67-0.93, 3,271 children). The current knowledge of mechanism that through which probiotics could reduce the
risk of eczema is not well understood. Furthermore, while no effect was observed for sensitisation in offspring (measured by SPT to any allergen) following pre/probiotic intake during pregnancy, the upper bound was very close to 1 and could be considered borderline (RR=0.91, 95% CI=0.82-1.02, 10 trials, 2,877 children). Overall, these analyses need to be viewed with caution given the risk of bias in included trials and statistical heterogeneity observed for some outcomes including eczema.

3.5.6.2. Overall completeness and applicability of evidence
There was a low quality of evidence that probiotic supplementation during pregnancy is effective for prevention of a number of allergic manifestations in the offspring. The meta-analysis for the outcome of “any eczema” also showed high statistical heterogeneity between the included trials ($I^2=66.2\%$). The heterogeneity between the trials limited the findings and random effect models were used to pool the results. Heterogeneity in the included trials resulted from varied dosage, mode of administration and type of probiotics as well as the timing and duration of interventions. There were also great differences between studies with regards to their sample size, duration of follow-up and diverse locations/settings.

Some evidence of publication bias was also observed from the visual inspection of funnel plot created for the outcome of “sensitisation, measured by SPT”.

3.5.6.3. Quality of evidence
Overall, the trials were at moderate to high risk of bias, for the summary of risk of bias assessment (Figure 3.2). A large proportion of studies were rated as unclear on many risk of bias domains and some had high risk of bias for individual quality domains (Figure 3.2). High loss to follow-up was rated as unclear and high risk of bias in 18 and 38% of studies respectively. Also the small size of the studies might have led to lack of precision which result in downgrading the quality of body of evidence.

3.5.6.4. Strength and weakness of this systematic review for pro/prebiotic consumption during pregnancy
The main distinction of this systematic review is that it includes only trials that have started intake of probiotics during pregnancy, thus, crucially, allows the effect of prenatal intake of probiotics for prevention of childhood allergies to be isolated. An
additional strength is that the systematic review followed an a priori published protocol, using a comprehensive search strategy that allowed for a complete coverage of all the relevant literature through citation databases, trial registries and conference proceedings. In addition, a range of allergic outcomes were considered for the present review and the most up-to-date results from the trials, reported as the longest available follow-up data, were included in the meta-analyses.

A limitation of the current review is that following the initial draft of the protocol some subsequent changes were made. Sub-group analyses were not conducted as planned since the effectiveness of prenatal and/or postnatal probiotics is investigated in the existing systematic reviews by various sub-group analyses. It is worth noting that sub-group analyses, conducted in these reviews, are mainly susceptible to type II errors due to relatively small sample sizes in trials and present a large statistical heterogeneity across the trials. However, with this exception, the established methodological guidelines from the protocol were followed, and there was a clear rational for deviations from the protocol. Consequently, data were pooled from trials conducted in diverse populations (infants with a family history of atopy and unselected samples) and using different probiotic formulations and doses and with a variety of quality.

3.5.6.5 Agreement and disagreement with other reviews

While the current systematic review was underway, two systematic reviews on the effectiveness of probiotics for prevention of allergies in offspring were published (Cuello-Garcia et al., 2015; Zuccotti et al., 2015). These reviews had a similar scope to that of the current systematic review investigating a range of allergic outcomes in children. However there are a number of key differences between this systematic review and that of Cuello-Garcia et al. (2015) and Zuccotti et al. (2015). Firstly, both of these reviews had different inclusion criteria where they have included studies that administered probiotics either prenatally (continued or not after birth) or only postnatally. Whereas, the present review only included studies that administered probiotics during pregnancy (and where continued, but not commenced postnatally). Secondly, both reviews had a limited searching where Cuello-Garcia and colleagues (2015) have excluded the extended follow-up reports for some of the unique RCTs included in their systematic review i.e. Abrahamsson et al (2013); Wickens et al.
(2013) and furthermore two other trials by Allen et al. (2014) and Gorissen et al. (2014) are not identified by the reviewers. The extended follow-up data from Dotterud et al. (2010) trial, reported by Simpson et al. (2015), was published after the release of Cuello-Garcia et al. (2015) review and has therefore not been included in their review. The review by Zuccotti et al. (2015) included 10 of the 13 original trials included in the present systematic review, two trials not identified (Kuitunnen et al., 2009 and Allen et al., 2014) and one excluded since it used a milk-based probiotic (Dotterud et al., 2010). Thirdly, the reviews by Cuello-Garcia et al. (2015) and Zuccotti et al., (2015) included both the earlier reports and/or reports on a sub-sample of an original RCT accompanied by the report of its longer-term follow-up in a number of conducted meta-analyses. Examples for the review by Cuello-Garcia and colleagues (2015) are Kukkonen et al. (2007) as the earlier report of Kuitunen et al. (2009) and Marschan et al. (2008), which is a report on a sub-sample of its original trial by Kukkunen et al. (2007). For the Zuccotti et al., (2015) review are Wickens et al., at 2008, 2012 and 2013 and Abrahamsson et al., at 2007 and 2013 in the same meta-analysis conducted. The current systematic review however, included only the latest available data, and where possible cumulative prevalence rather point prevalence, that reported the extended follow-up from their original RCTs on prenatal intake of probiotics. Furthermore, the Cuello-Garcia et al., (2015) and Zuccotti et al., (2015) reviews conducted sub-group analyses for the probiotic strain, follow-up duration and sample separately for pregnant women only, breast-feeding mothers only, infants only and pregnant with/without breast-feeding mothers with/without infants. However, sub-group met-analyses were not conducted in the existing systematic review. Moreover, both reviews by Cuello-Garcia et al. (2015) and Zuccotti et al., (2015) review have combined the wheeze/asthma together in meta-analyses whereas separate meta-analyses for wheeze and asthma outcomes were conducted in this review. In addition, studies with more than one intervention arm are included separately in meta-analyses in the previously conducted reviews i.e. Rautava et al., (2012) and Wickens et al., (2013) and more importantly, they did not split the number of participants in the control group for each intervention arm. But in the current review, the study arms were combined and compared versus the placebo group. Finally, in this review meta-analyses for sensitisation, measured by sIgE and SPT, were conducted and these analyses were not addressed in the reviews by Cuello-Garcia et al. (2015) and Zuccotti et al., (2015).
There are also key differences in the scope and approach of the existing systematic review with the other related systematic reviews, listed in section 3.3.2.2. With the exception of one review by Doege et al. 2012 that included only studies with prenatal intake of probiotics, all other reviews included both studies that administered probiotics either prenatally or only postnatally. Furthermore, all these reviews have focused on selected allergic outcomes. It is also important to note that these reviews have not included the follow-up data of many original RCTs and only the earlier reports of RCTs are included in the meta-analyses such as Kuitunen et al. (2009) and Kalliomäki et al. (2007) trials. Furthermore, a number of previous systematic reviews have included both an original trial as well as a report on its sub-sample in the same meta-analysis (examples as above). The current systematic review only included the longest available follow-up data from included original RCTs in meta-analyses and additionally, a clear distinction was made between an original trial and its further reports, either on its sub-samples or earlier follow-ups and these are reported as the companion papers. The exception to this is where we included the earlier reports of three RCTs, as described in section 3.5.3 since the outcome of interest was only reported in these earlier reports (and not in the follow-up reports).

The above-mentioned discussion highlights some key limitations in the previously conducted systematic reviews on this topic jeopardising the confidence that one can put in their results. Initially, they have failed to address the latest follow-up data for some of the included trials and thus to assess the longer-term effect of probiotic intervention. In addition, some of the unique RCTs were excluded from these reviews not enabling an evaluation of a comprehensive list of all the available studies conducted in the field. Another important issue is that the meta-analysis results of these reviews might have been biased by the fact that they have double or even triple counted one set of study participants in one analysis i.e. including the earlier and later reports from the same original trial, and consequently there is a risk that effects of intervention might have been overestimated in the analyses of these reviews (Sterne, Egger, & Moher, 2011).

The results from the present systematic review provides an update to the earlier systematic reviews of pro/prebiotics for prevention of childhood allergic diseases; nevertheless our results are not directly comparable to other reviews. Our results on
prenatal intake of probiotics, including the most up-to-date follow-up data from included trials in the meta-analysis, are in agreement with the results of existing systematic reviews indicating that consumption of probiotics might reduce the risk of childhood eczema. In our review, a broad definition of eczema was considered, atopic and non-atopic, and therefore a bigger RR and wider 95% CI was observed in comparison with that of the reviews by Cuello-Garcia et al. (2015) and Zuccotti et al., (2015) (RR=0.79, 95% CI=0.67-0.92 vs. RR=0.71, 95% CI=0.59-0.84 and RR=0.78, 95% CI=0.69-0.89), respectively. Our findings did not support that intake of pro/prebiotics during pregnancy effected the development of other allergic outcomes similar to the findings reported in other reviews. Our results also did not provide evidence that prenatal intake of pro/prebiotics affected the development of sensitisation (measured by positive SPT to any allergen) in children; however the upper boundary of 95% CI is very close to 1 (RR=0.91, 95% CI=0.82-1.02). Pooled analysis of a review conducted by Zhang et al., (2016); including prenatal and postnatal probiotic interventions, reported a reduced risk of sensitisation in children as measured by either SPT or sIgE and the effect is more significant when including studies that supplemented probiotics prenatally. It is worth adding that the reviewers did not make a distinction between sensitisation diagnosed by SPT or sIgE, and where there has been a report for both tests in the included trials, they have considered the results for SPT only in their analysis. This might limit the interpretation of these findings in practice as to which test might have better precision for assessing sensitisation.

3.5.6.6. Author’s conclusion

Implications for practice

This systematic review found that there are no positive effects of prenatal probiotic consumption for reducing the risk of development of most of the allergic outcomes. However, there is some evidence that suggests probiotic consumption during pregnancy could reduce the risk of developing all types of eczema in children and might also protect against childhood sensitisation. Nevertheless, the evidence from the current body of literature is not conclusive due to the risk of bias, different methods for reporting the outcome measures across studies, timing of intervention, variability of the probiotics and dosage used, and the diverse locations/settings.
Implications for research

Taking the quality of the available evidence into account, the effect of probiotic intake during pregnancy for prevention of childhood allergies needs to be further investigated in well-designed and executed RCTs.

Optimal timing of probiotic intervention is a fundamental factor that needs to be addressed in further trials. Most of the conducted trials have administered the intervention later in pregnancy. The immunomodulatory effects of probiotics are well recognised where they promote a healthy immune system development, and metabolic regulation in foetus (Nylund et al., 2013). This highlights the prominence of early interventions and the notion of using probiotics in pre-pregnancy and as early as pregnancy to optimise maternal and foetal microbiome. In addition, the transmission of probiotic strains might vary where a study showed that only a specific strain of probiotic was detectable in infant stool samples (Dotterud et al., 2015). The uncertainties regarding the choice of probiotics, dose, timing, mode of administration as well as the ideal duration of intervention requires further investigation. It is worth mentioning that with the exception of one study by Kuitunen et al., (2009) that used a combination of pre and probiotics, all other included trials in the current review administered probiotics only as the intervention. The efficacy of prebiotics and probiotics for prevention of childhood allergies, additionally, need to be differentiated in future trials.

Well-planned, multi-centre, coordinated RCTs with large samples designed to measure and report the outcomes in a consistent way, and focusing on different aspects including type of study sample i.e. atopic vs. non-atopic could provide stronger evidence regarding the impact of these dietary interventions during pregnancy on allergic outcomes in the offspring. Also, the majority of the studies were conducted in developed countries, and it remains a priority to understand the effectiveness of probiotics for prevention of allergic outcomes in offspring among the underreported populations.
3.5.7. Description of included studies of maternal fatty acid consumption during pregnancy and prevention of allergic diseases in the offspring

In total, seven studies (which included 2,492 children) examined the impact of fatty acid supplementation during pregnancy on the development of allergic diseases in offspring. The characteristics of the included trials, their companion papers and study population are presented in Table 3.5. Two studies were conducted in Australia and the rest were conducted in Mexico, Sweden, Finland, United Kingdom and Denmark.

The study conducted by Escamilla-Nuñez et al., (2014) had the largest sample size, randomising 1,094 pregnant women. This was followed by the Olsen et al., (2008) and Palmer et al., (2013) studies with 533 and 706 participants randomised respectively. The lowest sample sizes of 98 and 123 were respectively reported for the studies carried out by Dunstan et al., (2003a) and Noakes et al., (2012) respectively (Table 3.5).

The studies also varied in the length of their follow-up. The longest follow-up period was 16 years, reported in Olsen et al. (2008), followed by the Palmer et al. (2013) which reported a 3 year follow-up period. Studies conducted by Furuhjelm et al., (2011); Linnamaa et al., (2010) had a follow-up time of 2 years and the shortest follow-up period (one year) reported in the study by Dunstan et al. (2003a).

Of the total seven fatty acids studies, four were conducted amongst a population with a high risk of atopy (Dunstan, Mori, Barden, et al., 2003a; Furuhjelm et al., 2011; Noakes et al., 2012; Palmer et al., 2013) and the other three were conducted with an unselected sample (Escamilla-Nuñez et al., 2014; Linnamaa et al., 2010; Olsen et al., 2008). Eczema and asthma were the most frequently reported allergic outcomes in these studies. Also, while there was a later publication from a study published in 2010 (Linnamaa et al., 2010), data was extracted from the 2010 report since the follow-up paper (Linnamaa et al., 2013) only reported pre-clinical outcomes (Breast milk cytokine concentration in pg/ml).

Compliance with the intervention was measured by different methods, including total number of capsules actually consumed, women’s self-report of oily fish intake using food frequency questionnaires during pregnancy, telephone calls reminder(s) as well as assessing plasma concentration of LCPUFA either at birth or a few weeks after delivery.
<table>
<thead>
<tr>
<th>Primary article</th>
<th>Companion articles*</th>
<th>Country, enrolment period</th>
<th>Trial type</th>
<th>Name and number of study arms</th>
<th>No. of participants **</th>
<th>No. at last F-U ***</th>
<th>Time points measured</th>
<th>Age at last F-U</th>
<th>Sample: high risk of Atopy</th>
<th>Outcomes reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Dunstan et al., 2003a)</td>
<td>(Barden et al., 2004) (Barden, Dunstan, Beilin, Prescott, &amp; Mori, 2006) (Denburg et al., 2005) (Dunstan et al., 2003b) (Prescott, Barden, Mori, &amp; Dunstan, 2007) (Meldrum, D’Vaz, Dunstan, Mori, &amp; Prescott, 2011)</td>
<td>Australia 1999-2001</td>
<td>PC-RCT</td>
<td>Fish-oil supplement and control</td>
<td>98 mothers</td>
<td>83 (40 vs. 43)</td>
<td>12 months</td>
<td>1yr.</td>
<td>Yes</td>
<td>-Wheeze</td>
</tr>
<tr>
<td>(Olsen et al., 2008)</td>
<td>(Olsen et al., 1992)</td>
<td>Denmark 1989-90</td>
<td>PC-RCT</td>
<td>Fish oil vs. No oil vs. Olive oil</td>
<td>533 mothers</td>
<td>528 (263 vs. 129 vs. 136)</td>
<td>16yrs.</td>
<td>16yrs.</td>
<td>No</td>
<td>-Any Allergic disease(s)</td>
</tr>
<tr>
<td>(Linnamaa et al., 2010)</td>
<td>(Linnamaa et al., 2013)</td>
<td>Finland 2004-08</td>
<td>PC-RCT</td>
<td>Blackcurrant seed oil &amp; olive oil</td>
<td>322 mothers</td>
<td>177 (85 vs. 92)</td>
<td>3, 12 &amp; 24 months</td>
<td>2yrs.</td>
<td>No</td>
<td>-Eczema</td>
</tr>
<tr>
<td>(Furuhjelm et al., 2011)</td>
<td>(Furuhjelm et al., 2009)</td>
<td>Sweden 2003-05</td>
<td>PC-RCT</td>
<td>Omega-3 group or placebo</td>
<td>145 mothers</td>
<td>116 (53 vs. 63)</td>
<td>3, 6, 12 &amp; 24 months</td>
<td>2yrs.</td>
<td>Yes</td>
<td>-Eczema</td>
</tr>
<tr>
<td>Primary article</td>
<td>Companion articles+</td>
<td>Country, enrolment period</td>
<td>Trial type</td>
<td>Name and number of study arms</td>
<td>No. of participants</td>
<td>No. at last F-U***</td>
<td>Time points measured</td>
<td>Age at last F-U</td>
<td>Sample: high risk of Atopy</td>
<td>Outcomes reported</td>
</tr>
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<tr>
<td>(Noakes et al., 2012)</td>
<td>(Helmersson-Karlqvist et al., 2012; Miles et al., 2011; Urwin et al., 2014)</td>
<td>UK. No reported</td>
<td>RCT*</td>
<td>Salmon &amp; Control</td>
<td>123 mothers</td>
<td>86 (48 vs. 38)</td>
<td>Birth &amp; 6 months</td>
<td>6 months</td>
<td>Yes</td>
<td>-Specific IgE -Food Allergy -Allergic Rhinoconjunctivitis -SCORAD</td>
</tr>
<tr>
<td>(Palmer et al., 2013)</td>
<td>(Palmer et al., 2012)</td>
<td>Australia 2006-08</td>
<td>PC-RCT</td>
<td>N-3 LCPUFA &amp; control</td>
<td>700 mothers</td>
<td>638 (333 vs. 305)</td>
<td>12 &amp; 36 months</td>
<td>3 yrs.</td>
<td>Yes</td>
<td>-Any Allergic disease(s) -Eczema -Asthma -SPT (egg) -SPT (peanut)</td>
</tr>
<tr>
<td>Primary article</td>
<td>Companion articles+</td>
<td>Country, enrolment period</td>
<td>Trial type</td>
<td>Name and number of study arms</td>
<td>No. of participants</td>
<td>No. at last F-U***</td>
<td>Time points measured</td>
<td>Age at last F-U</td>
<td>Sample: high risk of Atopy</td>
<td>Outcomes reported</td>
</tr>
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</tr>
</tbody>
</table>
| (Escamilla-Nuñez et al., 2014) | (Ramakrishnan et al., 2010) (Imhoff-Kunsch et al., 2011) (Lee et al., 2014) | Mexico 2005-07 | PC-RCT | Docosahexaenoic acid or Placebo | 1,094 mothers | 869 (429 vs. 440) | 1, 3, 6, 9, 12 & 18 months | 18 months | No | -Wheezing
-Cough
-Breathing Difficulty
-Phlegm & Nasal Discharge
-Fever

*Published data and conference presentation, no unique data were extracted from conference abstracts
**Indicates the number at randomisation, where recruitment has occurred prenatally
***Follow-Up

Placebo Controlled-Randomised Controlled Trial
Single-blinded Randomised Controlled Trial
Table 3.6 shows the details of fatty acid interventions and placebo used in included trials. In all studies, the intervention and placebo groups had comparable baseline characteristics at recruitment and also birth.

Five studies administered the fatty acid supplements during pregnancy only (Dunstan, et al., 2003; Escamilla-Nuñez et al., 2014; Noakes et al., 2012; Olsen et al., 2008; Palmer et al., 2013) and, in the remaining two, fatty acid interventions were applied both during pregnancy and after delivery in mothers only (Furuhjelm et al., 2011) or in both mothers and infants (Linnamaa et al., 2010). The longest duration of intervention (30-32 months) was reported in the study by Linnamaa et al. (2010) and the shortest (2-2.5 months) by Olsen et al. (2008). Trials also differed in the timing of the introduction of the intervention, beginning as early as 8 weeks of gestation or as late as 30 weeks of gestation.

The nature of the fatty acid regimen during pregnancy was different across the included studies: n-3 (Omega-3) PUFA was used in two studies (Dunstan, et al., 2003a; Furuhjelm et al., 2011; Palmer et al., 2013); Docosahexaenoic Acid (DHA) was used in one study (Escamilla-Nuñez et al., 2014); Blackcurrant Seed Oil (BCSO) was used in one study (Linnamaa et al., 2010); one study used Fish Oil (Olsen et al., 2008) and the final study used salmon portions (Noakes et al., 2012). The composition of fatty acid supplements in the included studies were similar, based on presented data in the papers, but it was not clear how the fatty acid profiles of plasma lipids in pregnant women might differ between different applied fatty acids products. In all the included studies, the fatty acid preparations were delivered as capsules except the study by Noakes et al., 2012 that used Salmon portions.

The diversity of comparators between studies were as follows: olive oil in three studies (Dunstan et al., 2003a; Linnamaa et al., 2010; Olsen et al., 2008), a mixture of corn and soy oil (Escamilla-Nuñez et al., 2014), mainly the omega-6 PUFA Linoleic Acid (LA) (Furuhjelm et al., 2011), vegetable oil (Palmer et al., 2013) and standard diet (Noakes et al., 2012). The choices of comparator in the Olsen et al. (2008) study were defined as either “olive oil” or “no treatment” and for this review all the analyses conducted for the fatty acid component vs. olive oil. The Noakes et al., 2012
study which had “standard diet” as its comparator was not included in the meta-analysis as this was not similar in nature to the other comparators.
Table 3.6. Characteristics of fatty acid interventions in included trials

<table>
<thead>
<tr>
<th>Primary article</th>
<th>Comparable baseline characteristic</th>
<th>Participants receiving intervention</th>
<th>Timing of Intervention in pregnancy</th>
<th>Intake of intervention from/until*</th>
<th>Duration of intervention (months)**</th>
<th>Fatty Acid product ***</th>
<th>Placebo</th>
<th>Mode of delivery</th>
<th>Total daily dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dunstan, et al., 2003a</td>
<td>Yes</td>
<td>Prenatally in mothers</td>
<td>From 20wks</td>
<td>20wks to delivery</td>
<td>4.5-5</td>
<td>N-3 PUFA</td>
<td>Olive oil</td>
<td>Capsule</td>
<td>4 (1-g) per day</td>
</tr>
<tr>
<td>Olsen et al., 2008</td>
<td>Yes</td>
<td>Prenatally in mothers</td>
<td>Around 30wks</td>
<td>30wks to delivery</td>
<td>2-2.5</td>
<td>Fish oil</td>
<td>Olive oil &amp; no treatment</td>
<td>Gelatin capsule</td>
<td>1-g, 4 a day</td>
</tr>
<tr>
<td>Linnamaa et al., 2010</td>
<td>Yes</td>
<td>Prenatally in mothers &amp; postnatally in mothers &amp; infants</td>
<td>Between 8-16wks</td>
<td>8-16wks to 2yrs. postnatal</td>
<td>30-32</td>
<td>BCSO</td>
<td>Olive oil</td>
<td>Capsule</td>
<td>3 g/day, 6 capsules, oil drops in infants</td>
</tr>
<tr>
<td>Furuhjelm et al., 2011</td>
<td>Yes</td>
<td>Pre &amp; postnatally in mothers</td>
<td>From 25wks</td>
<td>25wks to 3.5months postnatal</td>
<td>7 to 7.5</td>
<td>Omega-3 PUFA</td>
<td>Mainly the omega-6 PUFA LA ***</td>
<td>Capsule</td>
<td>500-mg, nine a day</td>
</tr>
<tr>
<td>Noakes et al., 2012</td>
<td>Yes</td>
<td>Prenatally in mothers</td>
<td>20wks</td>
<td>20wks to delivery</td>
<td>4.5-5</td>
<td>Salmon Portions</td>
<td>Standard diet</td>
<td>-</td>
<td>2 portions per week</td>
</tr>
<tr>
<td>Palmer et al., 2013</td>
<td>Yes</td>
<td>Prenatally in mothers</td>
<td>From 21wks</td>
<td>21wks to delivery</td>
<td>4.5</td>
<td>N-3 PUFA</td>
<td>Vegetable oil</td>
<td>Capsule</td>
<td>500 mg, 3 per day</td>
</tr>
<tr>
<td>Escamilla-Nuñez et al., 2014</td>
<td>Yes</td>
<td>Prenatally in mothers</td>
<td>From 18 or 22wks</td>
<td>18-22wks until delivery</td>
<td>4.5-5.5</td>
<td>DHA</td>
<td>A mixture of corn and soy oil</td>
<td>Capsule</td>
<td>400 mg, twice per day</td>
</tr>
</tbody>
</table>

*Indicates total duration in mother, infant or both, whichever is applicable
**Indicates total duration in pregnancy plus after birth either in mother only or both mother and infants, if applicable
***DHA=Docosahexaenoic Acid, PUFA=Poly-Unsaturated Fatty Acid, BCSO=Blackcurrant Seed Oil, LA=Linoleic Acid
3.5.8. Risk of bias in studies of maternal fatty acid consumption during pregnancy and prevention of allergic diseases in the offspring

Figure 3.13 shows the summary of risk of bias assessment in trials on fatty acid studies. Most of the included studies were rated as low risk of bias for most domains. Two studies were rated as high risk (29%) in the double blinding domain. Appendix 3.7 shows the reviewer’s judgment of the risk of bias assessment in fatty acid studies.
<table>
<thead>
<tr>
<th>Study author</th>
<th>Random Sequence Generation</th>
<th>Allocation Concealment</th>
<th>Double Blinding</th>
<th>Blinding of Outcome Assessment</th>
<th>Incomplete Outcome Data</th>
<th>Selective Outcome Reporting</th>
<th>Other Sources of Bias</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dunstan (2003a)</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Olsen (2008)</td>
<td>?</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Linnamaa (2010)</td>
<td>+</td>
<td>?</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Furuhjelm (2011)</td>
<td>?</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Noakes (2012)</td>
<td>+</td>
<td>?</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Palmer (2013)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Escamilla-Nuñez (2014)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Random Sequence Generation
- Low risk of bias: 57%
- High risk of bias: 43%

Allocation Concealment
- Low risk of bias: 57%
- High risk of bias: 43%

Double Blinding
- Low risk of bias: 71%
- Unclear risk of bias: 29%

Blinding of Outcome Assessment
- Low risk of bias: 100%

Incomplete Outcome Data
- Low risk of bias: 43%
- High risk of bias: 57%

Selective Outcome Reporting
- Low risk of bias: 100%

Other Sources of Bias
- Low risk of bias: 100%

Figure 3.13. Summary of risk of bias assessment in the included trials of fatty acids and prevention of allergy in the offspring
3.5.8.1. Random sequence generation (selection bias)
Four studies were assessed as low risk of bias for their randomisation method. The remaining three studies were assessed as having an unclear risk of bias (Dunstan, et al., 2003a; Furuhjelm et al., 2011; Olsen et al., 2008) where it has been addressed as either block randomisation or generally stated as randomly assigned with no details on the method of randomisation.

3.5.8.2. Allocation concealment
The studies by Linnamaa et al. (2010) and Furuhjelm et al. (2011) were rated as ‘unclear’ as there was no information on allocation concealment. The study by Noakes et al. (2012) was also rated ‘unclear’ as it was a single-blind study with no reference to allocation concealment. Methods for concealment of allocation were reported in the remaining studies and were subsequently classified as having a low risk of bias for allocation concealment.

3.5.8.3. Double Blinding (performance bias)
Two studies were deemed as high risk for performance bias. The study by Olsen et al. (2008) provided no information on blinding of research staff and/or participants. Noakes et al., (2012) trial was a single-blind study and it was not specified whether the participants or the research staff were blinded. The rest of the studies were classified as low risk for their performance bias.

3.5.8.4. Blinding of outcome assessment (detection bias)
The detection bias was rated as low risk in all the included fatty acid trials since the assessment of outcome was conducted in a blind manner.

3.5.8.5. Incomplete outcome data (attrition bias)
lack of completeness of data was rated as low risk in three of the included trials as the reasons for attrition or exclusion were comparable across the study arms. Four studies rated as high risk of bias (Dunstan et al., 2003a; Furuhjelm et al., 2011; Linnamaa et al., 2010; Noakes et al., 2012). In the study by Dunstan et al. (2003) the loss to follow-up and discontinuation rates due to nausea were higher in the intervention arm. Loss to follow-up cases due to pregnancy-related nausea and poor compliance in both study arms were high (>50%) in the Linnamaa et al. (2010) trial. In Furuhjelm et al. (2011) the minimum 15 weeks of supplementation required was not met by 23% and 12% of women in the intervention and placebo arms respectively, and a higher loss to
follow-up rate was also observed in the intervention group. The study by Noakes et al. (2012) did not report the reasons for loss to follow-up.

3.5.8.6. Selective outcome reporting (reporting bias)
All the trials had reported the pre-specified outcomes in their published study protocol or published papers and were classified as low risk.

3.5.8.7. Other potential sources of bias
There were no concerns regarding any other sources of bias in the included trials and all were assessed as low risk.

3.5.9. Meta-analyses of effectiveness of maternal fatty acid consumption during pregnancy and prevention of allergic diseases in the offspring
Pooled results from meta-analysis in the studies that examined the effectiveness of fatty acid supplementations for prevention of allergic outcomes in offspring are presented in the following section. For some outcomes there was an insufficient number of studies and these have been presented descriptively. Also, the Noakes et al., (2012) was not included in meta-analysis, as its comparator was not similar to the control group in other included trials. The results from this study are described narratively.

3.5.9.1. Any ‘Allergic Diseases’ as an outcome measure for fatty acid intervention
The use of fatty-acid supplementations during pregnancy for the prevention of allergic diseases was assessed in three studies. Figure 3.14 shows the forest plot for fatty acids versus placebo in pregnant women for the prevention of allergic diseases in offspring. Any ‘allergic disease’ was defined differently in the included studies. These are described as below:

Furuhjelm et al. (2011): Symptoms of eczema, food reaction, asthma or rhinoconjunctivitis were considered as clinical symptoms of allergic diseases. This outcome was reported as point prevalence at 2 years.

Olsen et al. (2008): Allergic disease was defined as ‘allergic asthma, atopic dermatitis or allergic rhinitis’. This outcome was reported as cumulative prevalence at 16 years.

Palmer et al. (2013): The outcome considered was ‘allergic disease, without sensitisation’ and was defined as eczema, asthma, allergic rhinitis or food allergy. All data is based on analysis of 50 imputed datasets. This outcome was reported as
cumulative incidence based on 1 and 3 years assessments.

A large amount of statistical heterogeneity existed between the included studies ($\chi^2=5.24, P=0.07, \Gamma^2=61.8\%$). The result of meta-analysis did not show an association between maternal intake of fatty acids during pregnancy and prevention of any allergic diseases in the offspring (RR=0.74, 95% CI=0.45-1.22, 1,222 children).

When interpreting the Forest plot, it is worth noting that the only available data for the Palmer et al. (2013) study (which had the largest sample size) was subject to multiple imputations, due to significant missing data, affecting 50 complete data sets.

**Figure 3.14. Forest plot of fatty acids vs. placebo for any allergic diseases**

Measure: Binary: risk ratio

Heterogeneity: Q = 5.24; df = 2; p = 0.0729; I-squared = 61.8%; tau-squared = 0.117. Random effects model: 0.743 (0.451, 1.22)

<table>
<thead>
<tr>
<th>Outcome: Any allergic diseases</th>
<th>Fatty acids n/N</th>
<th>Placebo n/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Furuhjelm</td>
<td>14/54</td>
<td>21/63</td>
</tr>
<tr>
<td>Olsen</td>
<td>6/263</td>
<td>10/136</td>
</tr>
<tr>
<td>Palmer</td>
<td>124/368</td>
<td>117/338</td>
</tr>
<tr>
<td><strong>Subtotal</strong></td>
<td><strong>685</strong></td>
<td><strong>537</strong></td>
</tr>
</tbody>
</table>

The Palmer et al. (2013) study has also reported the outcome as ‘allergic disease, with sensitisation’. We did not pool the ‘allergic disease, with and without sensitisation’ together into meta-analysis in order to avoid double counting of cases with other allergic outcomes. We conducted a meta-analysis separately including the data for ‘allergic disease, with sensitisation’ rather than ‘allergic disease, without sensitisation’ for the Palmer et al. (2013) study (forest plot not shown) and this did not alter the pooled result in a significant manner (RR=0.70, 95 % CI=0.45-1.1).
3.5.9.2. Asthma as an outcome measure for fatty acid intervention

Four included studies measured the effect of fatty acid consumption during pregnancy on the prevention of asthma in offspring (Figure 3.15). The definition of asthma in the included trials in the meta-analysis were as below:

**Dunstan et al. (2003a):** The outcome considered was ‘Asthma’ and its diagnosis was made in children with recurrent wheezing (3 or more episodes, at least 1 confirmed by a pediatrician or general practitioner). All children diagnosed with asthma had to be responsive to bronchodilator therapy. This outcome was reported as point prevalence at 1 year.

**Furuhjelm et al. (2011):** The outcome considered was ‘Any asthma’. Asthma was defined as doctor diagnosed wheezing at least three times during the first 2 years of life. IgE-associated asthma was defined as asthma with the presence of IgE antibodies or positive SPT. This outcome was reported as cumulative incidence at 2 years.

**Olsen et al. (2008):** The outcome considered was ‘Allergic asthma’ and was diagnosed by the DJ450 code, using the International Coding of Diseases version 10 (ICD-10). Data were collected using the National Patient Registry (NPR), a mandatory national hospital discharge registry in Denmark that for many years has recorded virtually all discharge diagnoses for hospitalisations. This data might have been biased for asthma cases that did not warrant hospitalisations. This outcome reported as point prevalence at 16 years.

**Palmer et al. (2013):** The outcome considered was ‘Asthma with sensitisation’. Asthma was defined as a history of 3 or more episodes of wheeze with the episodes less than 6 weeks apart and/or daily use of asthma medication. This data was analysed by Fisher’s exact test using original data and adjusted/imputed analyses were not carried out due to rarity of outcomes. This outcome was reported as the cumulative incidence based on 1 and 3 year assessments.

There was a moderate level of heterogeneity between the included trials ($\chi^2=6.56$, P=0.86, $I^2=54.4\%$). The results of meta-analysis did not show an association between maternal intake of fatty acids during pregnancy for reducing the risk of asthma in offspring (RR=0.55, 95% CI=0.21-1.41, 1,307 children).
Figure 3.15. Forest plot of fatty acids vs. placebo for asthma

Measure: Binary: risk ratio
Heterogeneity: $Q = 6.59; \text{df} = 3; p = 0.0862; I^2 = 54.5\%; \tau^2 = 0.495$.
Random effects model: 0.552 (0.215, 1.41)

<table>
<thead>
<tr>
<th>Outcome: Asthma</th>
<th>Fatty acids n/N</th>
<th>Placebo n/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dunstan (2003)</td>
<td>2/40</td>
<td>6/43</td>
</tr>
<tr>
<td>Furuhjelm (2011)</td>
<td>7/54</td>
<td>8/65</td>
</tr>
<tr>
<td>Palmer (2013)</td>
<td>6/368</td>
<td>5/338</td>
</tr>
<tr>
<td><strong>Subtotal</strong></td>
<td><strong>725</strong></td>
<td><strong>582</strong></td>
</tr>
</tbody>
</table>

3.5.9.3. Eczema as an outcome measure for fatty acid intervention

The meta-analysis on the maternal consumption of fatty acids during pregnancy and its effectiveness on prevention of eczema in the offspring is shown in Figure 3.16. In total, four studies were included in the meta-analysis. Included trials used different definitions of eczema but for this systematic review, all the definitions were considered. The definition of eczema in the included trials in meta-analysis were as below:

**Dunstan et al. (2003a):** The outcome considered was ‘Atopic dermatitis’. The diagnosis of atopic eczema dermatitis syndrome was made in infants with typical skin lesions or physician-diagnosed eczema responsive to topical steroids. This outcome was reported as point prevalence at 1 year.

**Furuhjelm et al. (2011):** The outcome considered was ‘Any eczema’. Eczema was defined as reoccurring and itching eczematous, lichenified or nummular dermatitis, according to the definition by Seymour in 1987. If detectable IgE antibodies or a positive SPT was present, it was defined as IgE-associated eczema. This outcome was reported as cumulative incidence at 2 years.

**Linnamaa et al. (2010):** The outcome considered was ‘Atopic eczema’. Atopic dermatitis was defined as a chronic or relapsing itchy dermatitis with a characteristic morphology and distribution, based on the Hanifin criteria. Atopic dermatitis was also recorded if there was a history of chronic or relapsing itchy dermatitis with a typical
localization. This outcome was reported as point prevalence at 2 years.

**Palmer et al. (2013):** The outcome considered was ‘Eczema with sensitisation’. Eczema was defined according to the Hanifin criteria. IgE-associated eczema or atopic eczema was defined as eczema with sensitisation to at least one of the allergens assessed. The data is based on analysis of 50 imputed datasets. This outcome was reported as cumulative incidence based on 1 and 3 years assessments.

Statistically, studies were moderately homogenous ($\chi^2=5.35$, $P=0.14$, $I^2=43.9\%$). The results of meta-analysis showed that there is no association between maternal intake of fatty acids during pregnancy for prevention of eczema in the offspring ($RR=0.83$, 95% CI=0.63-1.12, 1,038 children) (Figure 3.16).

**Figure 3.16. Forest plot of fatty acids vs. placebo for eczema**

Measure: Binary: risk ratio
Heterogeneity: $Q = 5.35; df = 3; p = 0.148; I$-squared = 43.9%; tau-squared = 0.0388.
Random effects model: 0.836 (0.623, 1.12)

<table>
<thead>
<tr>
<th></th>
<th>Fatty acids n/N</th>
<th>Placebo n/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dunstan (2003)</td>
<td>18/40</td>
<td>13/43</td>
</tr>
<tr>
<td>Furuholm (2011)</td>
<td>11/54</td>
<td>21/65</td>
</tr>
<tr>
<td>Linnamaa (2010)</td>
<td>33/85</td>
<td>45/92</td>
</tr>
<tr>
<td>Palmer (2013)</td>
<td>51/368</td>
<td>64/338</td>
</tr>
<tr>
<td><strong>Subtotal</strong></td>
<td>547</td>
<td>538</td>
</tr>
</tbody>
</table>

**3.5.9.4. Wheeze as an outcome measure for fatty acid intervention**

In total, two studies were included in a meta-analysis measuring the effect of fatty acid intake during pregnancy on the prevention of wheeze in the offspring (Figure 3.17). The definition of wheeze in the included trials were as below:

**Dunstan et al. (2003a):** The outcome considered was ‘Recurrent wheeze’ and was defined on >2 occasions. This outcome was reported as point prevalence at 1 year.
**Escamila-Nuñez et al. (2014):** The outcome considered was ‘Wheezing, maternal atopy and non-atopy’. This study has reported wheezing separately for ‘atopic’ and ‘non-atopic’ mothers. We combined the two outcomes to allow for the broadest definition. The authors have also reported that the multivariate analysis for child’s sex, low birth weight and maternal education did not show a difference between wheezing status of children from non-atopic mothers compared to atopic mothers (p value=0.55). Using a clinical questionnaire, the detailed information about the presence or absence of signs and respiratory symptoms and the number and duration of episodes was provided. Symptomatic episode of wheeze was defined for each sign and symptom, coded as 1 for the presence of at least one symptom or sign lasting ≥3 days or 0 otherwise. The combination of the presence of various symptoms or signs at the same time was also considered. This outcome was reported as point prevalence at 1.5 years.

No statistical heterogeneity was observed between the included trials ($\chi^2=0.001$, P=0.97, I$^2=0$ %). There was no evidence that consumption of fatty acids during pregnancy, in atopic and non-atopic mothers, reduces the risk of wheezing in offspring (RR=0.97, 95% CI=0.82-1.15, 952 children).

**Figure 3.17. Forest plot of fatty acids vs. placebo for wheeze**

Measure: Binary: risk ratio  
Heterogeneity: Q = 0.00143; df = 1; p = 0.97; I-squared = 0%; tau-squared = 0.  
Random effects model: 0.973 (0.823, 1.15)

<table>
<thead>
<tr>
<th>Outcome: Wheeze</th>
<th>Fatty acids n/N</th>
<th>Placebo n/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dunstan</td>
<td>10/40</td>
<td>12/43</td>
</tr>
<tr>
<td>Escamilla-Nuñez</td>
<td>252/429</td>
<td>262/440</td>
</tr>
<tr>
<td><strong>Subtotal</strong></td>
<td><strong>469</strong></td>
<td><strong>483</strong></td>
</tr>
</tbody>
</table>

The Escamila-Nuñez et al. (2014) study had also reported outcomes on ‘wheezing, maternal atopy’ and ‘wheezing, maternal non-atopy’ and we performed meta-analyses including these two outcomes separately (Forest plots not shown). The analyses did
not substantially alter the pooled results either for children from atopic mothers (RR=0.88, 95% CI=0.66-1.17) or for children from non-atopic mothers (RR=1.02, 95% CI=0.83, 1.24).

3.5.9.5. Rhinitis as an outcome measure for fatty acid intervention
Rhinitis was reported only in one study (Palmer et al., 2013). Another study (Furuhjelm et al., 2011) presented data on ‘rhinoconjunctivitis, any’ as well as ‘IgE-mediated rhinoconjunctivitis’. Therefore no meta-analysis was performed and the data is presented narratively.

In Palmer et al. (2013), the outcome was reported as ‘Allergic rhinitis, with sensitisation’. Allergic rhinitis was defined as a 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. IgE-associated asthma/allergic rhinitis was defined as asthma/allergic rhinitis along with sensitisation to at least one of the aeroallergens tested. This outcome was reported as cumulative incidence based on 1 and 3 year assessments. The results for this outcome, based on analysis of 50 imputed datasets, showed that there were no differences between the n-3 LCPUFA and control groups in the percentage of children diagnosed with allergic rhinitis with sensitisation through the first 3 years of age (RR= 0.82, 95% CI=0.44–1.54).

3.5.9.6. Food allergy as an outcome measure for fatty acid intervention
In total three studies were included in a meta-analysis measuring the association between maternal intake of fatty acids during pregnancy and the risk of developing food allergy in offspring (Figure 3.18). Food allergy was defined as below:

**Dunstan et al. (2003a)**: Food allergy (undefined), this outcome was measured as point prevalence at 1 year.

**Furuhjelm et al. (2011)**: The outcome considered was ‘Any food reactions’. Food reactions were diagnosed by gastrointestinal symptoms, hives, aggravated eczema or wheezing following the ingestion of a certain food with recovery after food elimination and reoccurrence of symptoms after re-ingestion of the particular food. If food-specific-positive SPT or serum IgE antibodies were present, the food reaction was considered as IgE mediated. The outcome was reported as cumulative incidence at 2 years.
Palmer et al. (2013): The outcome considered was ‘Food allergy, with sensitisation’. IgE-associated food allergy was defined as a history of immediate (within 60 min) skin rash (hives, rash or swelling) with or without respiratory symptoms (cough, wheeze, stridor), gastrointestinal symptoms (abdominal pain, vomiting, loose stools) or cardiovascular symptoms (collapse) following ingestion of a food and sensitisation to the implicated food. The data is based on analysis of 50 imputed datasets. This outcome was reported as cumulative incidence based on 1 and 3 year assessments.

A moderate level of heterogeneity was observed between the included trials ($\chi^2$=3.02, $P=0.22$, $I^2=33.8\%$). The results of the Forest plot did not reveal an association between intake of fatty acids during pregnancy and the risk of developing food allergy in the offspring (RR=0.73, 95% CI=0.39-1.44, 908 children).

Figure 3.18. Forest plot of fatty acids vs. placebo for food allergy

Measure: Binary: risk ratio
Heterogeneity: $Q = 3.02; df = 2; p = 0.221; I$-squared = 33.8%; tau-squared = 0.113. Random effects model: 0.753 (0.394, 1.44)

<table>
<thead>
<tr>
<th>Outcome: Food allergy</th>
<th>Fatty acids n/N</th>
<th>Placebo n/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dunstan (2003)</td>
<td>3/40</td>
<td>5/43</td>
</tr>
<tr>
<td>Furuhjelm (2011)</td>
<td>6/54</td>
<td>16/65</td>
</tr>
<tr>
<td>Palmer (2013)</td>
<td>18/368</td>
<td>14/338</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>462</strong></td>
<td><strong>446</strong></td>
</tr>
</tbody>
</table>

3.5.9.7. Raised specific Immunoglobulin E (sIgE) as an outcome measure for fatty acid intervention

Any positive sIgE, as the outcome of interest for this systematic review, was not reported in any of the included studies. Therefore we could not conduct any meta-analysis for any positive sIgE as an outcome.
3.5.9.8. Positive SPT to any allergen as an outcome measure for fatty acid intervention

Three studies were included in a meta-analysis measuring the effect of fatty acids intake during pregnancy and sensitisation, as measured by SPT in the offspring (Figure 3.19). Sensitisation was measured by SPT and a positive reaction was described in the included trials as below:

**Dunstan et al. (2003a):** The outcome considered was ‘SPT, any positive’. The tests were performed using a standardised technique and allergen extracts (egg, milk, peanut, house dust mite, cat), as well as histamine as a positive control and glycerin as a negative control. A wheal diameter of ≥2 mm was considered positive. This outcome was reported as point prevalence at 1 year.

**Furuhjelm et al. (2011):** The outcome considered was ‘any positive SPT’. SPTs were performed in the infants at 6, 12 and 24 months of age to milk, egg, wheat and cat. At 24 months of age, timothy and birch were added to the test panel. Food allergens were tested prick-to-prick. A wheal diameter ≥3 mm was considered positive. The outcome is reported as cumulative incidence at 2 years.

**Palmer et al. (2013):** The outcome considered was ‘any positive SPT’. Sensitisation was defined as a positive skin prick test (wheal ≥3 mm above negative control) to at least one of the allergens assessed. At 1 year of age, the food allergens tested were whole hen’s egg, cow’s milk, wheat, tuna and peanut, and the aeroallergens tested were ryegrass pollen, olive tree pollen, *Alternaria tenuis*, cat hair and house dust mite (*Dermatophagoides farinae=Der p*). At 3 years of age, the same allergens were tested with the addition of two more foods (cashew nut and sesame seed) and one additional aeroallergen (house dust mite, *Der P*). The cow’s milk allergen extract became unavailable from the supplier for an extended period during the 3-year assessments and consequently was excluded from the definition of sensitisation at 3 years. The data is based on analysis of 50 imputed datasets. This outcome was reported as cumulative incidence based on 1 & 3 year assessments.

Statistically, studies were largely homogeneous with no variation between studies ($\chi^2=1.83$, $P=0.04$, $I^2=0\%$). Meta-analysis results suggest that intake of fatty acids in pregnant women is associated with a reduction of risk of sensitisation in offspring, as measured by SPT to any allergens (RR=0.78, 95% CI= 0.64-0.95, 891 children).
### Figure 3.19. Forest plot of fatty acids vs. placebo for SPT (any positive)

Measure: Binary: risk ratio

Heterogeneity: $Q = 1.83; \text{df} = 2; \ p = 0.401; \ I^2 = 0\%; \ tau^2 = 0$.

Random effects model: $0.788 \ (0.648, 0.958)$

<table>
<thead>
<tr>
<th>Outcome: Any Positive SPT</th>
<th>Fatty acids n/N</th>
<th>Placebo n/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dunstan</td>
<td>9/35</td>
<td>14/37</td>
</tr>
<tr>
<td>Furuhjelm</td>
<td>10/52</td>
<td>22/61</td>
</tr>
<tr>
<td>Palmer</td>
<td>108/368</td>
<td>119/338</td>
</tr>
<tr>
<td><strong>Subtotal</strong></td>
<td><strong>455</strong></td>
<td><strong>436</strong></td>
</tr>
</tbody>
</table>

#### 3.5.9.9. Anaphylaxis and angioedema as an outcome measure for fatty acid intervention

Anaphylaxis and angioedema were defined as the outcomes of interest for this systematic review and these are described narratively as they have been reported in only one study (Dunstan et al., 2003). The results from this study showed that fewer children in the fish oil group developed either anaphylaxis (0/40 (fish oil) vs. 1/43 (placebo)) or angioedema (1/40 (fish oil) vs. 5/43 (placebo)); these differences were not significant ($P>0.05$) and the small sample size limits confidence in these findings.

#### 3.5.9.10. Description of the outcomes reported in Noakes et al. 2012 study

This study used ‘salmon portion’ (as opposed to fatty acid supplements, as utilised in all the other included studies) compared to ‘standard diet’ during pregnancy for prevention of allergic disorders in offspring. The study reported a range of pre-clinical and clinical outcomes measured in offspring at 6 months age. The clinical outcomes of the interest for this systematic review are listed in Table 3.7. The results suggest that the use of salmon portions in pregnant women in comparison to standard diet did not have an influence on the prevention of atopic dermatitis and wheeze in the offspring by 6 months age; however, the small sample size limits confidence in these findings.
Table 3.7. List of the reported clinical outcomes in Noakes et al., 2012

<table>
<thead>
<tr>
<th></th>
<th>Control (n/N)</th>
<th>Salmon (n/N)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atopic dermatitis</td>
<td>7/38</td>
<td>12/48</td>
<td>0.46</td>
</tr>
<tr>
<td>Wheeze</td>
<td>7/37</td>
<td>11/46</td>
<td>0.58</td>
</tr>
</tbody>
</table>

3.5.10. Discussion of the evidence synthesis of fatty acid consumption during pregnancy and prevention of allergic diseases in the offspring

3.5.10.1. Summary of main results

The current systematic review summarised data from seven RCTs of maternal intake of fatty acids during pregnancy for prevention of allergic outcomes in children, including a total of 2,492 children with follow-up duration ranging from 6 months to 16 years (mean 3.7 years). Trials were at risk of bias with 29% and 57% trials rated high risk for detection and attrition bias respectively. Most trials had a small sample size and also the choice of fatty acids, control group, timing and duration of intervention as well as duration of follow-up was varied. The findings from this systematic review and meta-analysis do not provide evidence that intake of fatty acids during pregnancy could protect subsequent development of a number of childhood allergic diseases including asthma, eczema, wheeze and food allergy. However, the evidence does suggest that prenatal fatty acids might have a protective effect for developing sensitisation in offspring, as measured by SPT to any allergen (RR=0.788, 95% CI=0.648, 0.958, 3 trials, 891 children). In general, the results from this review need to be considered with caution because of the heterogeneity observed between the studies and risk of bias.

3.5.10.2. Overall completeness and applicability of evidence

There was a low quality of evidence that fatty acid supplementation during pregnancy is not effective for prevention of several allergic manifestations in the offspring. The meta-analysis for the outcome of “sensitisation, measured by SPT” did not show statistical heterogeneity between the included trials ($I^2=0\%$), however the number of studies included limits the results. The heterogeneity between the trials limited the findings and random effect models were used to pool the results. Heterogeneity between the included trials resulted from choice of fatty acids and the dose, timing and duration of interventions as well as duration of follow-up and small sample size
in most studies.

3.5.10.3. Quality of evidence
Overall, the trials were at moderate to high risk of bias, for the summary of risk of bias assessment (Figure 3.13). Randomisation and allocation concealment were deemed as unclear for 43% of included studies in each domain. High loss to follow-up was also a major concern where 57% of studies showed a high attrition bias and might have largely biased the effect of intervention within these trials. In addition, a limited number of seven studies fulfilled the inclusion criteria for the fatty acids intervention group and consequently, a small number of studies contributed in the conducted meta-analyses for most allergic outcomes.

3.5.10.4. Strength and weakness of this systematic review for fatty acid consumption during pregnancy
The key strengths and weaknesses of this systematic review for fatty acid consumption during pregnancy for prevention of childhood allergies are the same as those discussed for the probiotics intervention (see section 3.5.6.4).

3.5.10.5 Agreement and disagreement with other reviews
Subsequent to the start date of the present review, two further systematic reviews on the effectiveness of n-3 LCPUFA supplementation (either with or without arachidonic acid) for prevention of allergies in early childhood have been published (Best et al., 2016; Gunaratne et al., 2015). As in the present review, these reviews had a similar scope examining a range of allergic diseases in children; however there are key differences between the present systematic review and that of Gunaratne et al., (2015) and Best et al., (2016). Firstly, the Gunaratne et al. (2015) review had different inclusion criteria where it has included studies with prenatal or only postnatal administration of fatty acids. The review by Best and colleagues (2016) included trials in which supplementation was only commenced during pregnancy and identified five of the trials included in the current review. The present review only included studies that administered fatty acids during pregnancy, a total of seven studies, regardless of whether the supplementation was continued postnatally or not. Secondly, in Gunaratne and colleagues (2015) review, individual meta-analyses were performed for medically diagnosed allergies with or without IgE-mediated sensitisation whereas the review by Best et al., (2016) only investigated IgE-mediated allergies. We
included the broadest definition of allergic outcomes in the meta-analyses regardless of being IgE-mediated or not. Where an outcome was reported as both with and without sensitisation, these were not combined and the outcome reported without sensitisation considered. Thirdly, the reviews by Gunaratne et al. (2015) and Best et al., (2016) have only considered point prevalence of allergic outcomes in meta-analyses. In the present review, the cumulative incidence where reported were included in the meta-analyses and if not reported, reports on point prevalence were considered. Also, the review by Gunaratne et al. (2015) included the earlier as well as the extended follow-up of a trial in some meta-analyses, while Best and colleagues (2016) only included the earlier follow-up report of the included trials in their meta-analyses. The current review has selectively included the longest available follow-up measures in the meta-analyses.

Furthermore, the review by Gunaratne et al. (2015) conducted sub-group meta-analysis for duration of follow-up and a series of other allergic outcomes and included only one study in most meta-analyses. They have also not separated out the effect of different types of control in their analysis and included a study with a different comparator in the meta-analysis. The review by Best et al. (2016) did not perform sub-group analyses. In the present review, no sub-group meta-analyses were undertaken, mainly because of the small number of studies and also, one study with a different comparator (Noakes et al., 2012) was not included in meta-analyses and reported narratively. In addition, the review by Gunaratne et al. (2015) has combined the control groups of 'placebo and no oil' together for the study by Olsen et al. (2008) in their meta-analyses, while Best et al. (2016) review has not included this study in any meta-analyses. However, we have made the comparisons between the intervention and control group as ‘olive oil’ for Olsen et al. (2008) trial. Likewise the trial conducted by Linnamaa et al. (2010) is not included in the Gunaratne et al. (2015) and Best et al. (2016) reviews whilst we included trials that have used fatty acid products that are rich in n-3 LCPUFA. Therefore, the Linnamaa et al. (2010) trial that has used BCSO was included in our review. The composition of BCSO corresponds to the recommended optimal dietary intake n-3 and n-6 fatty acids with n-3/n-6 ratio from 1.3 to 1.4. Moreover, Gunaratne et al. (2015) have included reports of wheeze and asthma outcomes together and the Best et al. (2016) review did not conduct meta-analyses for either of these outcomes. The present review conducted
separate meta-analyses for wheeze and asthma in children. Finally, in the reviews by Gunaratne et al. (2015) and Best et al. (2016), most and all of the meta-analyses were conducted using a fixed-effect model. The current review, however, conducted all meta-analyses using a random-effect model considering all the differences between the included trials.

The current systematic review also had a different scope and approach from the existing systematic reviews, as described in section 3.3.2.2. Of the two existing systematic reviews, one has narratively described the studies that administered n-3 LUPUFA either prenatally or postnatally (Kremmyda et al. 2011). The review by Klemens and colleagues, (2011), including prenatal and postnatal trials of n-3 LUPUFA on selected allergic outcomes, does not include the most recent trials (Noakes et al., 2012; Palmer et al., 2013; Escamilla-Nuñez et al; 2014). This review has also excluded the study conducted by Linnamaa et al. (2010). In addition we aimed to include the most up-to-date available follow-up data from the included trials in the meta-analyses; however, this was not intended in the existing reviews and earlier follow-up data are included in some of the conducted meta-analyses.

The aforementioned detailed discussion features some key limitations in the previously conducted reviews on this topic, limiting the level of confidence in their findings. Most of the previously conducted reviews have failed to assess the evidence from the latest available follow-up data in the included trials of fatty acid interventions, administered either during pregnancy or after birth. Some of the unique RCTs are excluded from these reviews which limits investigating the evidence of all the conducted studies on this specific topic. As mentioned in section 3.5.6.4, the results of these reviews are also partly exposed to the risk of duplicating participants and, therefore, overestimating the effect of the intervention (Sterne, et al., 2011).

Due to the differences in focus between the above-mentioned systematic reviews and the current systematic review, the ability to make meaningful comparisons between the findings is to some extent limited. However, where comparisons are possible these will now be discussed. To recap, this systematic review examined prenatal intake of fatty acids only (that commenced prenatally and continued postnatally) including the most up-to-date follow-up data from included trials in the meta-analysis, indicating
that fatty acids did not protect against developing any types of eczema in children. In contrast, the reviews by Gunaratne et al. (2015) and Best et al. (2016) showed a beneficial effect for atopic eczema; however, these analyses only included the earlier follow-up data from included trials. The results of this systematic review also did not support the hypothesis that fatty acids could protect against other allergic outcomes in offspring. Similar results are reported in other systematic reviews, although the approaches in the conducted meta-analyses varied to some extent. The findings of this systematic review also showed a protective effect for childhood sensitisation measured by SPT to any allergen in line with the results in the other reviews. Meta-analyses for sensitisation to single allergens measured by SPT were not conducted in this systematic review, however, the reviews by Gunaratne et al. (2015) and Best et al. (2016) conducted meta-analyses for these outcomes and showed promising results. It is important to note that these meta-analyses only included a few studies and are subject to type II error.

3.5.10.6. Author’s conclusion

Implications for practice

The evidence on prenatal fatty acids intake during pregnancy and prevention of allergic outcomes in children is limited, although there appears to be an association with reduction of childhood sensitisation. There is a need to interpret these findings with caution as a result of risk of bias between studies, different methods for reporting the outcome measures, variability of the fatty acid supplements and doses as well as timing and duration of intervention and diverse settings.

Implications for research

Taking the quantity and quality of the available evidence into account, the effect of fatty acids intake during pregnancy for prevention of childhood allergies needs to be further investigated in well-designed and executed RCTs.

The timing of intervention has been varied in the conducted trials and the optimal timing of fatty acids intervention is an uncertainty that needs to be addressed more clearly in further trials. The n-3 LCPUFAs have anti-inflammatory properties that could alter the balance of $T_{H1}$ to $T_{H2}$, which is a well-recognised hallmark in allergies (Meydani et al., 1991; Thies et al., 2001; Trebble, et al., 2003). Therefore, it could be hypothesised that early introduction of fatty acids during pregnancy could promote a
beneficial immunomodulatory effect in the foetus as early as possible towards a healthy programming of the immune system. Furthermore, future trials could consider a differentiation between DHA to EPA ratios in fatty acid supplementation as well as baseline LCPUFA status in their sample. The dose and duration of intervention as well as the choice of control should also be addressed in future studies.

Large multicentre well-executed RCTs with coherent methodology and standardised measures for assessing the outcomes are required in order to make stronger inferences for the efficacy of fatty acids intervention in pregnant women as a preventive approach for childhood allergies. In addition, studies should aim to minimise the attrition bias in their extended follow-ups. There is also a need for well-designed epidemiological studies from under-reported populations, since the current evidence originates predominantly from the developed regions.
3.5.11. Description of included studies of maternal food avoidance interventions during pregnancy and prevention of allergic diseases in the offspring

A total of four included studies examined the impact of food allergen avoidance during pregnancy on the development of allergic disease in offspring (including a total of 634 children). The characteristics of the included trials and study populations for these studies are shown in Table 3.8. Two studies were conducted in Sweden, one in the United Kingdom and one in the United States (Table 3.8).

In one of the studies (Lilja et al., 1989) the sample is reported as consisting of two groups recruited from Stockholm and Uppsala. However, in their earlier report (Lilja et al., 1988), they had reported that the sample for their study was recruited from Stockholm and Uppsala as well as Linköping and a total of four diet interventions were concurrently taking place in the sample. For clarification, the authors were contacted and the reply is pasted below (personal communication):

“Two studies were started in Sweden around 1982-1983, one in Linköping (Falth Magnusson) and one in Stockholm-Uppsala (Lilja et al.). At that time avoidance was the main message to prevent allergy development in childhood. The women in Linköping were randomly allocated to a diet during the third trimester completely free from egg and milk or a "normal" intake and the women in Stockholm-Uppsala were randomly allocated to a reduced intake or a high intake of milk and egg. We decided to pool our data on the influence of the various maternal diets during late pregnancy on maternal and foetal IgG-antibody responses and on cord blood IgE responses in the paper published 1988 (Note: Data from 1988 paper is not extracted as it reports pre-clinical outcomes only). After the publication in 1988 we continued to include women in our studies and the results were published separately by Falth Magnussson and myself during the forthcoming years. Falth Magnusson has published a follow-up at 5 years of age. We have performed follow-ups at 5, 10 and now at 30 years of age. We will present our data from the follow up at 30 years of age at EAACI in Barcelona in June 2015.”

Based on the author’s reply, it was concluded that subsets of women initially allocated to different types of food allergen avoidance were reported in the later reports of Lilja’s trial. This was also considered in pooling the data from this study and the fact that the sample reported in Lilja study (1989) were also the sub-sets of sample reported in the 1992 study (Fälth-Magnusson & Kjellman, 1992).
Also in the Lilja et al., (1989) trial, after delivery two groups naturally emerged with respect to reduced diet group. One group of mothers (n=25) continued their restricted diet during their first two months of lactation by choice, whilst the rest of the mothers normalised their diet. These groups were called ‘reduced A and B’ groups and no significant differences were found between these groups with regards to SPTs or in the incidence of atopic diseases. Therefore, the results in the A-group and B-group were considered together and presented as the reduced group.

In the study conducted by Lovegrove, Hampton, & Morgan (1994), mothers allocated to the prophylactic-treated group were all atopic. However, mothers assigned to the un-restricted diet were grouped as atopic and non-atopic and allergic outcomes are presented separately for these groups. For this systematic review, the results from the atopic mothers with unrestricted diet are compared to prophylactic-treated group of atopic mothers.

The largest sample size was reported by the study conducted by Zeiger et al., (1992), which included 288 mothers at recruitment, followed by Fälth-Magnusson & Kjellman (1992) with a total of 209 pregnant women. The smallest sample was observed in the pilot study conducted by Lovegrove et al. (1994) with 44 pregnant mothers. In all the included studies, the sample was selected from a population with a family history of atopy.

Trials varied in their follow-up period from 1.5 years (Lilja et al., 1989 and Lovegrove et al., 1994) to 7 years (Zeiger et al., 1992). The data for the Zeiger et al (1992) study has been extracted from the report on 4 years follow-up (1992 paper) as the report for 7 years follow-up (Zeiger & Heller, 1995) has only presented the trends of allergic outcomes in children. While there was a later publication from the study by Lovegrove at el., (1996), only data from the 1994 report was extracted since the paper published in 1996 reported pre-clinical outcomes only e.g. levels of food antibodies and antigens in breast milk. This is also the case for the study by Lilja et al.; where only preclinical outcomes were reported in the 1991 report e.g. egg and milk-specific IgE and IgG antibodies. Therefore the clinical outcomes reported in the 1989 paper have been extracted for this review.
Table 3.8. Characteristics of included trials and study population for food avoidance interventions and prevention of allergy in offspring

<table>
<thead>
<tr>
<th>Primary article</th>
<th>Companion articles+</th>
<th>Country, enrolment period</th>
<th>Trial type</th>
<th>Name of the study arms</th>
<th>No. of participants</th>
<th>No. at last F-U***</th>
<th>Time points measured</th>
<th>Age at last F-U</th>
<th>Sample: high risk of Atopy</th>
<th>Feeding restriction s</th>
<th>Outcomes reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Lilja et al., 1989)</td>
<td>(Lilja et al., 1988, 1991)</td>
<td>Sweden</td>
<td>RCT</td>
<td>High diet vs. reduced diet</td>
<td>171 mothers</td>
<td>162 (79 vs. 83)</td>
<td>2, 6, 12 &amp; 18 months</td>
<td>1.5 yrs.</td>
<td>Yes</td>
<td>-</td>
<td>-Atopic eczema -Asthma -Rhinocconjunctivitis -Urticaria -SPT (egg) -SPT (cow’s milk)</td>
</tr>
<tr>
<td>(Zeiger et al., 1992)</td>
<td>(Zeiger et al., 1989; Zeiger &amp; Heller, 1995)</td>
<td>the US</td>
<td>RCT</td>
<td>Prophylactic-treated &amp; Control</td>
<td>288 mothers</td>
<td>225 (85 vs. 140)</td>
<td>4 months, 3, 4 &amp; 7** yrs.</td>
<td>7yrs.</td>
<td>Yes</td>
<td>BF encouraged or infant formulas used</td>
<td>-Any allergy -Atopic dermatitis -Food Allergy -Allergic rhinitis -Asthma -SPT (any positive) -SPT inhalant (any positive) -Urticaria</td>
</tr>
<tr>
<td>(Fälth-Magnusson &amp; Kjellman, 1992)</td>
<td>(Fälth-Magnusson &amp; Kjellman, 1987; Fälth-Magnusson, Oman, &amp; Kjellman, 1987)</td>
<td>Sweden 1983-4</td>
<td>RCT#</td>
<td>Exclusion Diet and None-exclusion Diet</td>
<td>209 mothers</td>
<td>209 (86 vs. 123)</td>
<td>3, 6, 12 &amp; 18 months + 5yrs.</td>
<td>5yrs.</td>
<td>Yes</td>
<td>-</td>
<td>-Allergic disease -Atopic dermatitis -Any food intolerance -Rhinocconjunctivitis -Asthma -Egg intolerance -Fish intolerance -SPT (any positive)</td>
</tr>
<tr>
<td>(Lovegrove et al., 1994)</td>
<td>(Lovegrove, Morgan, &amp; Hamptom, 1996)</td>
<td>the UK 1988-9</td>
<td>RCT# (Pilot study)</td>
<td>Prophylactic-treated (atopic) &amp; Unrestricted-diet (atopic &amp; non-atopic)</td>
<td>44 mothers</td>
<td>38 (12 vs. 14 vs. 12)</td>
<td>6, 12 &amp; 18 months</td>
<td>1.5 yrs.</td>
<td>Yes</td>
<td>Exclusive BF encourage d with late start for solids</td>
<td>-Atopic eczema -Severity of allergy -Maternal and cord serum β-Lg-IgG, α-cas-IgG and β-Lg</td>
</tr>
</tbody>
</table>

+Excludes conference abstracts from which no unique data were extracted
**Indicates the number at randomization where recruitment has occurred prenatally
*Single blind study

*Randomized Controlled Trial
***Follow-Up
**at 7yrs., only the trends of outcomes are reported
The characteristics of food allergen avoidance interventions and the control groups in the included trials are presented in Table 3.9. Comparable baseline characteristics have been reported in all the included trials.

The two Swedish studies, (Fälth-Magnusson & Kjellman, 1992; Lilja et al., 1989) had applied the food avoidance intervention only throughout pregnancy whilst, in the rest of the trials (Table 3.9) the intervention was also continued during the lactation period, with the longest period of intervention being 3 years postnatally in the Zeiger et al. (1992) study.

Trials varied in the nature of food avoidance in pregnant women from complete avoidance of dairy as well as peanut products in the study by Zeiger et al. (1992), to the avoidance of dairy products and egg in the rest of the included studies. The choice of control group in three of the studies was ‘standard diet’ (Fälth-Magnusson & Kjellman, 1992; Lovegrove, Hampton, & Morgan, 1994; Zeiger et al., 1992) and one study used a diet which included consumption of 1 hen’s egg and 1 litre of milk daily as the comparator arm (Lilja et al., 1989). The latter was not included in the meta-analysis as the control was very different to the other comparators and quite artificial.

With the exception of the trial conducted by Lilja et al. (1989), all participants randomised to the intervention arms were prescribed substitutes including calcium, vitamins and also formula for their infant to compensate for the nutrient deficiency that could be caused by restricting egg and dairy intake.

Compliance with the food allergen avoidance intervention in the included studies was assessed by either the mother’s daily records of their intake, or contacting women during the period of intervention. There was no indication of how compliance with the intervention was monitored in the trial by Fälth-Magnusson & Kjellman (1992).
<table>
<thead>
<tr>
<th>Primary article</th>
<th>Comparable baseline characteristic</th>
<th>Participants receiving intervention</th>
<th>Timing of Intervention in pregnancy</th>
<th>Intake of intervention From/until</th>
<th>Duration of intervention (months)**</th>
<th>Type of food intervention</th>
<th>Control arm</th>
<th>Substitutes prescribed (in Int arm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lilja et al., 1989</td>
<td>Yes (but a significant higher incidence of atopic eczema and higher IgE levels before 28wks in the ‘reduced’ group)</td>
<td>Pre &amp; postnatally in mothers (after delivery, on mothers’ own choice)</td>
<td>From 28wks</td>
<td>28wks to delivery</td>
<td>2.5-3</td>
<td>Strictly reduced ingestion of egg and dairy products (milk/yoghurt/butter/cheeses etc.)</td>
<td>High diet (1 hens’ egg and dairy products corresponding to about 1L of cows' milk daily)</td>
<td>-</td>
</tr>
<tr>
<td>Zeiger et al., 1992</td>
<td>Yes</td>
<td>Prenatally in mothers &amp; postnatally in infants</td>
<td>During the last trimester of pregnancy</td>
<td>3rd trimester to 3yrs. postnatal*</td>
<td>36-40</td>
<td>Avoidance of all cow's milk, egg, and peanut products</td>
<td>Standard diet (recommended by ACOG)</td>
<td>1500mg daily prenatal vitamins &amp; calcium</td>
</tr>
<tr>
<td>(Fälth-Magnusson &amp; Kjellman 1992)</td>
<td>No (Tobacco exposure was statistically significant between the D &amp; ND groups)</td>
<td>Prenatally in mothers</td>
<td>From 28wks</td>
<td>28wks to delivery</td>
<td>2.5-3</td>
<td>Avoidance of Dairy Product &amp; Egg (Cow's milk and egg)</td>
<td>Standard diet (1/2 litre of milk/day &amp; 3-5 eggs/wk. extra calcium and casein hydrolysate (Nutramigen) supplement)</td>
<td>-</td>
</tr>
<tr>
<td>Lovegrove et al., 1994</td>
<td>Yes</td>
<td>Pre &amp; postnatally in mothers</td>
<td>From approximately 36wks</td>
<td>36wks to lactation period</td>
<td>4.5-5 (majority breast-fed by 4 months)</td>
<td>Avoidance of all milk and dairy products</td>
<td>Standard diet (for pregnancy &amp; lactation)</td>
<td>Hypoallergenic complete infant formula, Peptijunioras required + 1000mg Ca supplement</td>
</tr>
</tbody>
</table>

*Mothers have continued food avoidance at different time points e.g. Late start of solids

**Indicates total duration in pregnancy plus after birth, if applicable
3.5.12. Risk of bias in studies of maternal food avoidance interventions during pregnancy and prevention of allergic diseases in the offspring

The summary of risk of bias of trials on food avoidance intervention studies is presented in Figure 3.20 In all the included studies, the allocation concealment was rated as unclear and all studies were rated as having a high risk of bias for their double blinding domain.

The reviewer’s judgment on the risk of bias assessment on food avoidance intervention studies is shown in appendix 3.8.
<table>
<thead>
<tr>
<th>Short Title</th>
<th>Random Sequence Generation</th>
<th>Allocation Concealment</th>
<th>Double Blinding</th>
<th>Blinding of Outcome Assessment</th>
<th>Incomplete Outcome Data</th>
<th>Selective Outcome Reporting</th>
<th>Other Sources of Bias</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lilja (1989)</td>
<td>?</td>
<td>?</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Zeiger (1992)</td>
<td>+</td>
<td>?</td>
<td>-</td>
<td>?</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Random Sequence Generation: 25% Low risk of bias, 75% High risk of bias
Allocation Concealment: 100% Low risk of bias
Double Blinding: 100% Low risk of bias
Blinding of Outcome Assessment: 50% Low risk of bias, 50% High risk of bias
Incomplete Outcome Data: 50% Low risk of bias, 50% High risk of bias
Selective Outcome Reporting: 50% Low risk of bias, 50% High risk of bias
Other Sources of Bias: 25% Low risk of bias, 25% High risk of bias, 50% Unclear risk of bias

Figure 3.20. Summary of risk of bias assessment in the included trials of food avoidance and prevention of allergy in the offspring.
3.5.12.1. Random sequence generation (selection bias)
One study was rated as having a low likelihood of selection bias (Zeiger et al., 1992), as they randomised the participants by using a computer system. Method of assignment was rated as unclear in three studies since there was no clear information (Fälth-Magnusson & Kjellman 1992; Lilja et al., 1989; Lovegrove et al., 1994).

3.5.12.2. Allocation concealment
All the food avoidance intervention trials failed to report any information for their allocation concealment and so were rated as having an unclear risk of bias.

3.5.12.3. Double blinding (performance bias)
All of the four studies were classified as having a high risk of bias, clearly stating that either blinding did not remain intact throughout the study or that it was not possible to blind the participants due to the nature of the intervention.

3.5.12.4. Blinding of outcome assessment (detection bias)
Only two studies described any blinding of outcome assessment and were rated as low risk (Lilja et al., 1989 & Lovegrove et al., 1994). The remainder were rated as unclear risk (Fälth-Magnusson & Kjellman 1992; Zeiger et al, 1992).

3.5.12.5. Incomplete outcome data (attrition bias)
Reasons for missing and/or exclusion of data were balanced across the study arms in two studies and therefore rated as low risk (Lilja et al., 1989 and Lovegrove et al. 1994). Two other studies were rated as high risk of bias since, as in the trial by Fälth-Magnusson & Kjellman (1992), some women had switched from the intervention arm to the control group and vice versa. The study by Zeiger et al (1992) also had a large rate of loss to follow-up by 4 years, largely in its intervention arm.

3.5.12.6. Selective outcome reporting (reporting bias)
In all the included trials, the pre-specified outcomes including the cumulative prevalence of allergic outcomes were reported; thus all trials were rated as low risk for selective outcome reporting.

3.5.12.7. Other potential sources of bias
Any further sources of bias within the included trials were considered and two studies were assessed as high risk (Fälth-Magnusson & Kjellman 1992; Lilja et al., 1989). In
both studies, some women decided to continue their diet during the breast-feeding period by choice and also some switched between the study arms. The study by Zeiger et al. (1992) was rated as having a low risk of bias. The study by Lovegrove et al. (1994) was rated as unclear as the trial was a pilot study and generously supported by Cow & Gate, Trowbridge, Witlshire, UK, as a commercial funding body.

3.5.13. Meta-analyses of effectiveness of maternal food avoidance interventions during pregnancy and prevention of allergic diseases in the offspring

Pooled results from meta-analyses in the studies that examined the effectiveness of food avoidance interventions during pregnancy for prevention of allergic outcomes in the offspring are presented in the following section. For the trial by Fälth-Magnusson & Kjellman (1992) the allergic outcomes at 5 years follow-up were analysed on the basis of compliance with diet rather than on the basis of randomisation. Also, the Zeiger et al. (1992) trial presented the cumulative prevalence of some outcomes by 4 years of age only in graph format. Where possible, numbers have been calculated from the data presented in these graphs, although there is the potential for this to have introduced a small amount of error since these are estimated rather than absolute data and discrepancies may have arisen during rounding. It was not possible to perform any meta-analysis for the outcomes ‘wheeze’ and ‘positive sIgE’ as these were not reported in any of the included trials. The Lilja et al. (1989) study was not included in meta-analyses, as its comparator was not similar to the control group in other included trials. The results from this study are described narratively.

3.5.13.1. Any ‘Allergic Diseases’ as an outcome measure for food avoidance intervention

The effectiveness of food avoidance during pregnancy for prevention of allergic diseases in the offspring was assessed in two studies. Figure 3.21 shows the Forest plot for food avoidance versus standard diet in pregnant women for the prevention of allergic diseases in offspring. ‘Any allergic disease’ was defined differently in the included studies. These are described as below:

Fälth-Magnusson & Kjellman (1992): The outcome considered was ‘Allergic diseases, definite’. Based on all available information about each child, a summarised classification regarding allergic disease was made by the authors. Children classified
as ‘allergic’ had a history that suggested allergy, verified by a positive SPT for the suspected allergen, and/or a diagnosis of allergic disease by a pediatrician. There is no information whether the outcome is calculated as point or cumulative prevalence at 5 years.

**Zeiger et al. (1992):** Any allergy was defined by phenotypes of allergy as physician documented lower respiratory asthma, atopic dermatitis, urticaria and allergic rhinitis plus sensitisation. This was reported as a cumulative prevalence at 4 years follow-up.

Statistically, the studies were largely homogeneous ($\chi^2=0.23$, $P=0.63$, $I^2=0\%$). The results of meta-analysis did not support a beneficial effect for food avoidance in pregnant women for prevention of allergic diseases in the offspring (RR=0.99, 95% CI=0.73-1.35, 486 children).

**Figure 3.21. Forest plot of food avoidance interventions vs. standard diet for any allergic diseases**

Measure: Binary: risk ratio
Heterogeneity: $Q=0.232; df=1; p=0.63$; I-squared = 0%; tau-squared = 0.
Random effects model: 0.999 (0.737, 1.35)

The Fälth-Magnusson & Kjellman (1992) study has also presented data on allergic diseases based on parental reports of all allergies. The number of children reported by parents manifesting allergic diseases in the diet group vs. the non-diet group were as follows: no allergy (45/84 vs. 67/114), mild allergy (28/84 vs. 30/114), moderate allergy (10/84 vs. 17/114) and severe allergy (1/84 vs. 0/114). There were no significant differences between the diet and non-diet groups. The parents of seven children reported allergic symptoms affecting skin, eye/nose, and bronchi, three in the diet group and four in the non-diet group respectively. The combined evaluation of all
available information, assessed by the authors about each child, corresponded well to
the parental opinion. The cases reported as ‘no allergy’ and ‘probable allergy’, as
assessed by the authors in the diet and non-diet groups were 55/84 vs. 72/114 and
5/84 vs. 12/114 respectively, and there were no significant differences between study
groups. The children evaluated as having ‘definite allergy’ by authors were included
in the meta-analysis (Figure 3.21).

3.5.13.2. Asthma as an outcome measure for food avoidance intervention
Two included studies measured the effect of food avoidance(s) during pregnancy on
prevention of asthma in offspring (Figure 3.22). The definition of asthma prevalence
in included studies included in the meta-analysis were as follows:

Fälth-Magnusson & Kjellman (1992): The outcome considered was ‘Obvious
bronchial asthma’. Parents of 52 children reported prolonged cough (>2 weeks after a
respiratory tract infection), or wheezing upon infections, or upon physical exercise at
least on one occasion. Parents were also offered a visit at clinic with a specialised
pediatric allergy nurse to discuss the questionnaire replies. Further clarification was
then sought as appropriate. Families, who declined the visit were interviewed on the
phone. Children considered to have allergic disease, severe enough to require
medication, were referred for a medical examination by a pediatrician. This was
performed in 47 children referred by the nurse. To evaluate bronchial obstruction, a
simple running exercise test was performed for 6 minutes according to the clinical
praxis of the department. A fall in the peak expiratory flow rate of >15% was
considered enough to verify a suspicion of asthma. This outcome was reported as
cumulative prevalence at 5 years.

Zeiger et al. (1992): Asthma was defined as a physician documented lower
respiratory condition with characteristics reversible bronchospasm, occurring at least
twice and un-associated with other anatomic, congenital, or immunologic causes. The
outcome was reported as cumulative prevalence at 4 years.

The results of the pooled analysis for asthma risk reduction in offspring following
food avoidance intervention during pregnancy are shown in Figure 3.22. There was a
moderate level of statistical heterogeneity between studies ($\chi^2=1.93$, $P=0.16$,
$\Gamma^2=48.1\%$). The pooled results from included studies did not support an association
between food avoidance during pregnancy and prevention of asthma in the offspring (RR=1.09, 95% CI=0.62-1.91, 486 children).

Figure 3.22. Forest plot of food avoidance intervention vs. standard diet for asthma

Measure: Binary: risk ratio
Heterogeneity: Q = 1.93; df = 1; p = 0.165; I-squared = 48.1%; tau-squared = 0.0829

Random effects model: 1.09 (0.628, 1.91)

<table>
<thead>
<tr>
<th>Outcome: Asthma</th>
<th>Food Int. n/N</th>
<th>Placebo n/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fälth-Magnusson (1992)</td>
<td>15/84</td>
<td>14/84</td>
</tr>
<tr>
<td>Zeiger (1982)</td>
<td>25/103</td>
<td>51/185</td>
</tr>
<tr>
<td>Subtotal</td>
<td>187</td>
<td>299</td>
</tr>
</tbody>
</table>

3.5.13.3. Eczema as an outcome measure for food avoidance intervention

Three included studies measured the effect of food avoidance during pregnancy on prevention of eczema in offspring (Figure 3.23). The definition of eczema prevalence in included studies in the meta-analysis were as follows:

Fälth-Magnusson & Kjellman (1992): The outcome considered was ‘Atopic dermatitis, adapted from the reports of the parents’. For diagnosing AD, the classification system proposed by Hanifin was used. This outcome was reported as point prevalence at 4 years follow-up. One point to consider is that there was a discrepancy between the numbers reported for this outcome (51 in the table and 53 in the text). We used the data provided in the table.

Zeiger et al. (1992): The outcome considered was ‘Atopic dermatitis’ and was defined as an eczematosus eruption associated with at least 3 of the following 4 criteria suggested by Hanifin and modified for offspring of atopic parents: i) pruritis, ii) typical morphology and distribution, iii) a tendency towards chronicity or recurrence, iv) concurrent specific-IgE. This was reported as cumulative prevalence at 4 years follow-up.

Lovegrove et al. (1994): The outcome considered was ‘Allergy incidence’ which was defined as the occurrence of clinically diagnosed atopic eczema. The outcome was
reported as point prevalence at 18 months. The results for atopic-diet vs. atopic unrestricted diet groups were considered.

The results of the pooled analysis for asthma risk reduction in offspring following food avoidance intervention during pregnancy are shown in Figure 3.23. There was a moderate level of statistical heterogeneity between studies ($\chi^2=6.35$, $P=0.04$, $I^2=68.5\%$). The pooled results from included studies did not support an association between food avoidance during pregnancy for prevention of eczema in offspring (RR=0.96, 95% CI=0.49-1.88, 512 children).

Figure 3.23. Forest plot of food avoidance intervention vs. standard diet for eczema

Measure: Binary: risk ratio
Heterogeneity: Q = 6.35; df = 2; $p = 0.0419$; I-squared = 68.5%; tau-squared = 0.234.
Random effects model: 0.962 (0.492, 1.88)

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Food Int. n/N</th>
<th>Placebo n/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Falth-Magnusson</td>
<td>14/84</td>
<td>12/114</td>
</tr>
<tr>
<td>Lovegrove</td>
<td>4/12</td>
<td>7/14</td>
</tr>
<tr>
<td>Zeiger</td>
<td>15/103</td>
<td>39/185</td>
</tr>
<tr>
<td>Total</td>
<td>199</td>
<td>313</td>
</tr>
</tbody>
</table>

In the study conducted by Lovegrove et al., 1994, three infants in the atopic-diet group inadvertently received at least one feed of commercial infant formula, derived from cow’s milk, shortly after delivery and developed allergic manifestations. We conducted another meta-analysis for this outcome in the atopic-diet group by excluding these children from the analysis. The result from the second meta-analysis showed a high heterogeneity ($\chi^2=8.89$, $P=0.01$, $I^2=77.5\%$) and there was no evidence that food avoidance protects children from developing eczema (RR=0.79, 95% CI=0.30-2.05) (Forest plot not shown).
3.5.13.4. Rhinitis as an outcome measure for food avoidance intervention

Rhinitis was reported only in one trial (Zeiger et al., 1992), and Fälth-Magnusson & Kjellman (1992) reported allergic rhinoconjunctivitis. Therefore, it was not possible to conduct a meta-analysis for this outcome.

The Zeiger et al. (1992) study defined the outcome of ‘Allergic rhinitis’ as a nasal condition with characteristic sneezing, itching, and/or rhinorrhea with concurrent i) sIgE and ii) nasal eosinophilia. This outcome was reported as cumulative prevalence at 4 years follow-up. Their results showed that there were no differences between the food allergen avoidance and standard diet groups in terms of the number of children diagnosed with allergic rhinitis through the first 4 years of life (43/104 vs. 24/185, p>0.05).

3.5.13.5. Food allergy as an outcome measure for food avoidance intervention

Two studies were included, which examined the effectiveness of food avoidance in pregnant women and prevention of food allergy in offspring (Figure 3.24). The definition of food allergy in the included studies, as described in the papers, were as follows:

Fälth-Magnusson & Kjellman (1992): The outcome considered was ‘Food intolerance, to any food item’. The parents were asked about signs and symptoms of food intolerance and whether the child had received any medication for allergic disease(s). There is no information on whether the outcome reported was point or cumulative prevalence at 5 years.

Zeiger et al. (1992): The outcome considered was ‘Food allergy, adverse allergic reactions to two or more foods’. Food allergy was defined when food-specific IgE occurred concurrently with symptoms consistent with: i) atopic dermatitis ii) urticaria/angioedema triggered at least twice by a specific food iii) gastrointestinal allergy (vomiting/diarrhea induced on at least two occasions by a specific food. DBPCFC were planned to confirm all non-anaphylactic adverse food reactions, but only half of these affected families agreed to undergo this procedure. The outcome was reported as point prevalence at 4 years.

Statistically, there was no heterogeneity between the included studies ($\chi^2 = 0.33$, P=0.56, $I^2 = 0\%$). The result of meta-analysis indicates that there was no association
between food avoidance during pregnancy and prevention of food allergy in the offspring (RR=1.04, 95% CI=0.51-2.07, 423 children).

**Figure 3.24. Forest plot of food avoidance intervention vs. standard diet for food allergy**

Measure: Binary: risk ratio  
Heterogeneity: $Q = 0.338; df = 1; p = 0.561; I^2$-squared = 0%; $tau$-squared = 0. 
Random effects model: 1.04 (0.518, 2.07)

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Food Int. n/N</th>
<th>Placebo n/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fälth-Magnusson</td>
<td>16/84</td>
<td>20/114</td>
</tr>
<tr>
<td>Zeiger</td>
<td>1/85</td>
<td>3/140</td>
</tr>
<tr>
<td>Subtotal</td>
<td>169</td>
<td>254</td>
</tr>
</tbody>
</table>

We also conducted a separate meta-analysis including the ‘cumulative prevalence’ of food allergy at 4 years of age, reported in Zeiger et al. (1992) study. The results from the meta-analysis showed a high heterogeneity between the included studies ($\chi^2=5.22, P=0.02, I^2=80.8\%$) showing no effect of prenatal food allergen avoidance in preventing food allergies in offspring (RR=0.66, 95% CI=0.25-1.75) (Forest plot not shown).

3.5.13.6. Positive Skin Prick Test (SPT) to any allergen as an outcome measure for food avoidance intervention

Two studies were included in a meta-analysis measuring the effect of food avoidance during pregnancy and sensitisation, measured by SPT in the offspring (Figure 3.25). Sensitisation was measured by SPT and a positive result was described in the included trials as below:

**Fälth-Magnusson & Kjellman (1992):** The outcome considered was ‘SPT, any positive’. The tests were performed according to the puncture method with a lancet with 1mm tip. The aeroallergens used were pasteurised cow’s milk with protein content and egg and milk extracts. The SPTs to milk and egg were performed in duplicates. Single pricks were performed to wheat, birch, timothy, cat, mites
(Dermatophagoides farina and D. pteronyssinus), mould (cladosporium) with Phazet. Histamine chloride, 10 mg/ml, as positive control. The wheal was marked on the skin with a filter-tipped pen and the mark was then covered with a transparent tape. The tape was later attached to a paper for measuring the reprint of the wheal. The SPT was regarded as positive when the mean wheel diameter was at least 3 mm. The outcome was reported as point prevalence at 5 years.

Zeiger et al. (1992): The outcome considered was ‘SPT, multiple foods’. Skin prick tests were with multi-test units using the food allergens as ‘milk, egg, peanut, wheat, corn, soy, cod, chicken, beef and other foods incriminated in a historical adverse reaction and aeroallergens ‘mite, alternaria, cladosporium (hornodndrum), grass mix (30% Bermuda, 70% grass mix), dog and cat’. SPT allergen wheel of ≥3 mm larger than the saline control were considered positive. Histamine phosphate was used for a positive control. The outcome was reported as period prevalence at 4 years.

Statistically, these studies are largely homogeneous with no variation between studies ($\chi^2=0.10$, $P=0.74$, $I^2=0\%$). The result of meta-analysis did not show an association between food avoidance during pregnancy and prevention of sensitisation in the offspring, measured by SPT to any allergens (RR=0.66, 95% CI=0.40-1.09, 443 children).

Figure 3.25. Forest plot of food avoidance intervention vs. standard diet for sensitisation to any allergen, measured by SPT

Measure: Binary: risk ratio
Heterogeneity: Q = 0.108; df = 1; p = 0.743; I-squared = 0%; tau-squared = 0.
Random effects model: 0.668 (0.409, 1.09)

<table>
<thead>
<tr>
<th>Outcome: Any Positive SPT</th>
<th>Food Int. n/N</th>
<th>Placebo n/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Falth-Magnusson</td>
<td>12/64</td>
<td>24/91</td>
</tr>
<tr>
<td>Zeiger</td>
<td>7/103</td>
<td>21/185</td>
</tr>
<tr>
<td>Subtotal</td>
<td>167</td>
<td>276</td>
</tr>
</tbody>
</table>

148
3.5.13.7. Narrative description of the results in the Lilja et al. (1989b) study

This study by Lilja et al. (1989) used food avoidance defined by strict reduction of ingestion of egg and dairy products (milk/yoghurt/butter/cheese etc.), and compared this arm with a high diet (one hen’s egg and 1L cow’s milk daily) during pregnancy for prevention of allergic disorders in the offspring. The study reported pre-clinical and clinical outcomes measured in offspring by 18 months of age. The clinical outcomes of interest for this systematic review are listed in the Table 3.10. As the results indicate, avoidance of specific foods in pregnant women in comparison to a diet rich in dairy products did not influence the risk of atopic dermatitis and asthma in the offspring.

Table 3.10. List of the reported clinical outcomes in Lilja et al (1989)

<table>
<thead>
<tr>
<th>Outcome</th>
<th>High diet (n/N)</th>
<th>Reduced Food Avoidance (n/N)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atopic eczema (obvious)</td>
<td>10/81</td>
<td>14/82</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Atopic eczema (probable)</td>
<td>3/81</td>
<td>4/82</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Atopic eczema (possible)</td>
<td>14/81</td>
<td>5/82</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Asthma (obvious)</td>
<td>1/81</td>
<td>1/82</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Asthma (probable)</td>
<td>0/81</td>
<td>0/82</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Asthma (possible)</td>
<td>3/81</td>
<td>6/82</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

3.5.14. Discussion of the evidence synthesis of food avoidance interventions during pregnancy and prevention of allergic diseases in the offspring

3.5.14.1. Summary of main results

The present systematic review summarised data from four RCTs of maternal food avoidance during pregnancy for prevention of allergic outcomes in children, including a total of 634 children with follow-up duration from ranging 1.5-7 years (mean 3.25 years). Trials were at risk of bias with all trials rated as unclear for their allocation concealment. In addition, performance bias was high risk in all trials (100%) while detection bias was deemed as low (50%) and unclear (50%) risk. High attrition bias was also a concern in two studies (50%). All trials had a small sample size and one was conducted as a pilot study (Lovegrove et al., 1994). The choice of food avoidance was fairly similar between studies, and only one study defined a different comparator. The findings from this systematic review and meta-analysis do not support a protective effect for food avoidance in pregnant women as an intervention strategy for prevention of allergic diseases in children. It should be noted that the results from this review are not decisive because of the low quality of studies as well as high
heterogeneity between studies.

3.5.14.2. Overall completeness and applicability of evidence

No evidence was found that food avoidance during pregnancy is effective for the prevention of allergic disorders in the offspring. The heterogeneity between the trials limited the findings and random effect models were used to pool the results. Heterogeneity between the included trials resulted from their sample size, duration of intervention, choice of control and follow-up duration.

3.5.14.3. Quality of evidence

Overall, the trials were at high risk of bias, for the summary of risk of bias assessment (Figure 3.20). Randomisation was rated as unclear in three studies (75%). All studies were open label due to the nature of intervention, resulting in high risk of bias for performance. High loss to follow-up was also a major concern in two studies (50%). In addition, there were few studies that met the inclusion criteria for this systematic review of food avoidance and, as a result, a relatively small number of studies was included in most of the meta-analyses.

3.5.14.4. Strength and weakness of this systematic review for food avoidance during pregnancy

The key strengths and weaknesses of this systematic review for food avoidance during pregnancy for prevention of childhood allergies are the same as those discussed for the probiotics intervention (see section 3.5.6.4).

3.5.14.5 Agreement and disagreement with other reviews

The current systematic review had a different approach than that of the existing systematic review conducted by Kramer & Kakuma (2012). Firstly, the review by Kramer and Kakuma (2012) had different inclusion criteria where they included trials on maternal dietary antigen avoidance in pregnant or lactating women, excluding trials of multimodal interventions that included manipulation of the infant’s diet other than breast milk or of non-dietary aspects of the infant’s environment. Therefore they have excluded the study by Zeiger et al. (1992) from their review. The current review included trials that started food allergen avoidance only during pregnancy (and where continued, but not commenced postnatally). Secondly, Kramer and Kakuma (2012) included one study where the available information is solely based on a published
abstract (Appelt et al. 2004); but this study is excluded from the present review. Thirdly, the review by Karmer and Kakuma (2012) included the follow-up data at 18 months for the study conducted by Fälth-Magnusson & Kjellman rather than data at 5 years. The reviewers justified that the longer-term follow-up results in this study are based on diets actually followed by women, rather than those allocated by randomisation. We included the 5 years follow-up knowing that the women in Fälth-Magnusson & Kjellman (1992) study were not blinded to their allocated group, and some switched between the groups during pregnancy and/or lactation. We agree that this is a breach of the study protocol and this is highlighted in the assessment of risk of bias. Furthermore, Kramer and Kakuma (2012) included the study by Lilja et al. (1989b) in their meta-analyses and in most of the analyses both the Lilja et al. (1989) and Fälth-Magnusson & Kjellman (1992) trials are included. We did not include the study by Lilja et al. (1989) in meta-analyses since the study defined a different comparator to the other included studies i.e. ‘high diet’ vs. ‘standard diet’. Also, the sample reported in this study was a sub-set of the sample from the Linköping area, which was the same sample reported by the Fälth-Magnusson & Kjellman trial. In addition, Kramer and Kakuma (2012) have performed most of the meta-analysis using a fixed-effect model, whereas we have conducted all the meta-analyses using a random-effect model reflecting all the variations between the trials. Finally, Kramer and Kakuma (2012) did not include the study by Lovegrove et al. (1994) in meta-analyses since it continued into lactation and was reported on its own. This study was included in one relevant meta-analysis in our review.

The results from this systematic review on food avoidance interventions during pregnancy for prevention of childhood allergies, including the latest available follow-up data from the included trials, indicated that food avoidance did not protect against developing allergies in the offspring i.e. any allergic diseases, asthma, eczema, food allergy and sensitisation. There were substantial differences between the analyses conducted in the present systematic review than that of the Kramer and Kakuma (2012) review, which makes the comparison of the findings restricted.

3.5.14.6. Author’s conclusion

Implications for practice
There is no evidence that food avoidance during pregnancy could prevent the development of allergic outcomes in children. The studies were very heterogeneous and had a high risk of bias.

**Implications for research**

In the 1980s, food avoidance of highly allergenic foods in pregnant women, particularly in atopic women, was believed to be the principal strategy for primary prevention of childhood allergies; however, this approach was mainly based on theories rather than evidence. This theory has been questioned during the recent decade(s) after trials on food elimination diet during pregnancy consistently failed to show a beneficial effect for prevention of childhood allergies (Fälth-Magnusson & Kjellman 1992; Lilja et al., 1989; Zeiger et al., 1992), accompanied by the rising trend of allergies and specifically higher prevalence of food allergies (Prescott, 2013). The open question nowadays is whether early exposure to allergenic foods in pregnancy could be a more effective approach for prevention of childhood allergies and emerging evidence from observational studies suggests this hypothesis could be true (Bunyavanich et al., 2014; Frazier, et al., 2014; Young, 2015). These findings provide an avenue for further research, exposure to allergenic foods as opposed to avoidance, by high-quality and well-executed trials.
3.5.15. Description of included studies with maternal vitamin consumption during pregnancy and prevention of allergic diseases in the offspring

A total of five RCTs examined the impact of vitamin supplementation during pregnancy on the development of allergic diseases in offspring, including 2,456 children. The characteristics of the included trials, their companion papers and study population are presented in Table 3.11. The trials were conducted across Europe in the United Kingdom, Netherlands and Denmark with two conducted in the United States.

The longest follow-up period was 3 years in studies conducted by Chawes et al., 2016; Goldring et al., 2013; Litonjua et al., 2016 and the shortest follow-up for 2 and 1 years reported respectively in studies by Greenough, Shaheen, Shennan, Seed, & Poston, 2010; McEvoy et al., 2014. The largest study sample was reported in Greenough et al. (2010) with 2,404 mothers enrolled, followed by Litonjua et al. (2016) study with 880 pregnant mothers at randomisation. The smallest sample sizes were observed in studies conducted by Goldring et al. (2013) and McEvoy et al. (2014) with 180 and 179 women randomised respectively.

The Chawes et al. (2016) study initially invited a larger sample of pregnant women to the trial and more than half declined to participate. A comparison of women who participated and not participated showed that participating women were characterised by higher prevalence of asthma, eczema and hay fever. Also, these women were more frequently employed as professionals and had higher income levels.

The sample studied in the included trials varied and included unselected pregnant women (Chawes et al., 2016), pregnant women with a history of atopy (Litonjua et al., 2016), pregnant women at risk of developing pre-eclampsia (Greenough et al., 2010), an unselected sample from different ethnic groups (Goldring et al., 2013) and smoking pregnant women (McEvoy et al., 2014). The most frequently reported allergic outcomes were wheeze and eczema.

Compliance with the intervention was measured by different methods, including counting of returned pills, dividing the number of capsules taken by the total number
prescribed in a given period, electronic medication container caps and telephone calls during the course of pregnancy to all women in the intervention arm.
Table 3.11. Characteristics of included trials and study population for vitamins and prevention of allergy in offspring

<table>
<thead>
<tr>
<th>Primary article</th>
<th>Companion articles</th>
<th>Country, enrolment period</th>
<th>Trial type</th>
<th>Name and No. of study arms</th>
<th>No. of participants **</th>
<th>No. at last F-U***</th>
<th>Time points measured</th>
<th>Age at last F-U</th>
<th>Sample: high risk of Atopy</th>
<th>Outcomes reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Greenough, et al., 2010)</td>
<td>(Poston, Briley, Seed, Kelly, &amp; Shennan, 2006)</td>
<td>Netherlands 2003-05</td>
<td>PC-RCT</td>
<td>Vitamins C &amp; E supplementation vs. Placebo</td>
<td>2,404 mothers</td>
<td>752 (386 vs. 366)</td>
<td>24 months</td>
<td>2yrs.</td>
<td>No</td>
<td>-Wheeze -Eczema -Asthma -Cough -Breathing Difficulty</td>
</tr>
<tr>
<td>(Goldring et al., 2013)</td>
<td>(Yu, Sykes, Sethi, Teoh, &amp; Robinson, 2009)</td>
<td>the UK 2007-unknown</td>
<td>RCT</td>
<td>Vitamin D supplements (as a daily dose &amp; single dose) vs. no treatment</td>
<td>180 mothers</td>
<td>158 (56 vs. 52 vs. 50)</td>
<td>36 months</td>
<td>3yrs.</td>
<td>No</td>
<td>-Wheeze -Eczema -Food allergy -Rhinitis -Atopy -URTIf -LRTIf -Inhaled bronchodilator or steroid</td>
</tr>
<tr>
<td>(McEvoy et al., 2014)</td>
<td>McEvoy 2013 (abstract conference)</td>
<td>the US 2007-11</td>
<td>PC-RCT</td>
<td>Vitamin C vs. placebo</td>
<td>179 mothers</td>
<td>159 (76 vs. 83)</td>
<td>Birth &amp; 12 months</td>
<td>1yr</td>
<td>No</td>
<td>-Wheeze -Breathing difficulty</td>
</tr>
<tr>
<td>(Chawes et al., 2016)</td>
<td>(Bisgaard et al., 2013; Bisgaard, 2004)</td>
<td>Denmark 2008-2010</td>
<td>PC-RCT</td>
<td>Vitamin D3 vs. placebo</td>
<td>623 mothers</td>
<td>581 (295 vs. 286)</td>
<td>Birth &amp; 36 months</td>
<td>3yrs</td>
<td>No</td>
<td>-Persistent wheeze -Asthma -SPT -sIgE -URTIf -LRTIf -Episodes of lung symptoms</td>
</tr>
<tr>
<td>(Litonjua et al., 2015)</td>
<td>(Litonjua et al., 2015)</td>
<td>US</td>
<td>PC-</td>
<td>Vitamin D vs.</td>
<td>880</td>
<td>806 (405)</td>
<td>Birth &amp; 36</td>
<td>3yrs</td>
<td>Yes</td>
<td>-Asthma or</td>
</tr>
<tr>
<td>Primary article</td>
<td>Companion articles</td>
<td>Country, enrolment period</td>
<td>Trial type*</td>
<td>Name and No. of study arms</td>
<td>No. of participants**</td>
<td>No. at last F-U***</td>
<td>Time points measured</td>
<td>Age at last F-U</td>
<td>Sample: high risk of Atopy</td>
<td>Outcomes reported</td>
</tr>
<tr>
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<tr>
<td>2016) al., 2014)</td>
<td>2009-2011</td>
<td>RCT</td>
<td>placebo</td>
<td></td>
<td>vs. 401)</td>
<td>months</td>
<td></td>
<td></td>
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<td>recurrent wheeze</td>
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<td></td>
<td>-Eczema with rash</td>
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<td>-LRTI</td>
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<td></td>
<td>-Total IgE</td>
<td>-Total IgE</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-Sensitisation (aeroallergens)</td>
<td>-Sensitisation (aeroallergens)</td>
</tr>
</tbody>
</table>

*Published data and no unique data were extracted from abstracts

**Indicates the number at randomisation, where recruitment has occurred prenatally

***Follow-Up

##Upper Respiratory Tract Infection

##Lower Respiratory Tract Infection
Table 3.12 shows the details of vitamin interventions and placebo applied in the included studies. Comparable characteristics have been reported in all the trials.

Four studies had used the vitamin supplementation only during pregnancy from as early as second trimester towards the end of second trimester of pregnancy (Table 3.12). In the trial by Chawes et al. (2016), women had started the intervention from 24 gestation weeks and continued after delivery for 1-week. The duration of intervention varied from 3.5-4 months to 7.5 months within the included trials.

The type of vitamin supplementations within the included studies were Vitamins C & E (Greenough et al., 2010), Vitamin D (Chawes et al., 2016; Goldring et al., 2013; Litonjua et al., 2016) and crushed Vitamin C (McEvoy et al., 2014). The Goldring et al. (2013) study had two intervention arms, as women were randomised either to receive a daily dose of ergocalciferol or a single oral dose of cholecalciferol (bolus). For the purpose of this systematic review, we have made the comparisons for both the daily dose of Vitamin D and combined Vitamin D groups. The trials by Chawes et al. (2016) and Litonjua et al. (2016) also supplemented women in both study arms with an extra 400 IU dose of multivitamin/Vitamin D3, as part of their routine care. The trial of Goldring et al. (2013) was originally conducted in the UK between April to November 2007, prior to the introduction of national guidance on Vitamin D intake during pregnancy in the UK in March 2008.

The choice of placebo in the Greenough et al. (2010) and McEvoy et al. (2014) trials were microcrystalline and ground starch respectively. The choice of control in the Goldring et al. (2013) was “no treatment” and the study has not been included in meta-analysis because of the inconsistency with the other comparators. The nature of placebo was not stated in the two studies by Chawes et al. (2016) and Litonjua et al. (2016).

In all trials, supplementation with vitamins significantly increased the serum concentration of vitamins in the intervention group compared to the control group.
Table 3.12. Characteristics of vitamin interventions in included trials

<table>
<thead>
<tr>
<th>Primary article</th>
<th>Comparable baseline characteristic</th>
<th>Participants receiving intervention</th>
<th>Timing of Intervention in pregnancy</th>
<th>Intake of intervention From/until</th>
<th>Duration of intervention (months)*</th>
<th>Vitamin product</th>
<th>Placebo</th>
<th>Mode of delivery</th>
<th>Total daily dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Greenough et al., 2010</td>
<td>Yes</td>
<td>Prenatally in mothers</td>
<td>From the 2nd trimester of pregnancy</td>
<td>From the 2nd trimester of pregnancy to delivery</td>
<td>6-6.5</td>
<td>Vitamin C &amp; E</td>
<td>Vitamin product cellulose with addition of tartaric &amp; citric acid + sunflower seed oil</td>
<td>Tablet</td>
<td>1000mg Vit C &amp; 400 IU RRR α-tocopherol, daily</td>
</tr>
<tr>
<td>Goldring et al., 2013</td>
<td>Yes</td>
<td>Prenatally in mothers</td>
<td>From 27wks to delivery</td>
<td>3months + 1 week</td>
<td>Vitamin D (cholecalciferol) or Vitamin D (ergocalciferol)</td>
<td>No treatment</td>
<td>Tablet</td>
<td>Single oral dose of 200,000 IU (bolus) or 800 IU daily</td>
<td></td>
</tr>
<tr>
<td>McEvoy et al., 2014</td>
<td>Yes</td>
<td>Prenatally in mothers</td>
<td>Randomized at 22wks or less</td>
<td>22wks to delivery</td>
<td>4-4.5</td>
<td>Crushed vitamin C</td>
<td>Ground corn starch</td>
<td>Capsule</td>
<td>500 mg, daily</td>
</tr>
<tr>
<td>Chawes et al., 2016</td>
<td>Yes</td>
<td>Pre &amp; postnatally in mothers</td>
<td>From week 24 after delivery</td>
<td>3.5-4 + 1 week</td>
<td>Vitamin D3 (cholecalciferol)</td>
<td>Tablets containing no active substance</td>
<td>Tablet</td>
<td>2400 IU, twice a day</td>
<td></td>
</tr>
<tr>
<td>Litonjua et al., 2016</td>
<td>Yes</td>
<td>Prenatally in mothers</td>
<td>Between 10-18wks</td>
<td>Between 12-18wks</td>
<td>7-7.5</td>
<td>Vitamin D &amp; placebo</td>
<td>Not mentioned</td>
<td>Tablets</td>
<td>4000 IU, daily</td>
</tr>
</tbody>
</table>

*Indicates total duration in pregnancy plus after birth, if applicable
3.5.16. Risk of bias in studies of maternal vitamin consumption during pregnancy and prevention of allergic diseases in the offspring

Figure 3.26 shows the summary of risk of bias assessment for the included trials on vitamin supplementations. Two studies were assessed as having high risk of bias for their double blinding domain whilst the allocation concealment and blinding of outcome assessment were ranked as low risk in all the included studies. The reviewer's judgment on the risk of bias assessment on vitamin studies is shown in appendix 3.9.
<table>
<thead>
<tr>
<th>Short Title</th>
<th>Random Sequence Generation</th>
<th>Allocation Concealment</th>
<th>Double Blinding</th>
<th>Blinding of Outcome Assessment</th>
<th>Incomplete Outcome Data</th>
<th>Selective Outcome Reporting</th>
<th>Other Sources of Bias</th>
</tr>
</thead>
<tbody>
<tr>
<td>Greenough (2010)</td>
<td>?</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>?</td>
</tr>
<tr>
<td>Goldring (2013)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>McEvoy (2014)</td>
<td>?</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Chawes (2016)</td>
<td>+</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>?</td>
</tr>
<tr>
<td>Litonjua (2016)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

**Figure 3.26. Summary of risk of bias assessment in the included trials of vitamins and prevention of allergy in the offspring**
3.5.16.1. Random sequence generation (selection bias)
Computer generated random numbers were reported in Goldring et al. (2013) and Chawes et al. (2016) trials, therefore these studies were rated as having a low risk of bias. The study by Litonjua et al. (2016) was also rated as low risk of bias as the randomisation was performed by the Data Centre Coordinating, using a system that automates the random assignment of treatment groups to study ID numbers by employing stratified permuted blocks with randomly varied block sizes. The two other studies were assessed as having an unclear risk since they stated randomisation with/without blocking without any further information.

3.5.16.2. Allocation concealment
There is no indication of allocation concealment in McEvoy et al. (2014) and Chawes et al. (2016) trials and hence these were rated as unclear. All the other trials had used an appropriate method for concealment of allocation and were rated as having a low risk of bias for allocation concealment.

3.5.16.3. Double blinding (performance bias)
Performance bias was assessed as high risk in two trials (Goldring et al., 2013; Greenough, et al., 2010). In Goldring et al. (2013), it was not possible to blind participants or investigators as participants would know if they had no treatment, daily tablets or a single bolus. The staff and parents were un-blinded at the extended follow-up of infants at 22 months in Greenough et al. (2010). The other three studies were rated as low risk of bias.

3.5.16.4. Blinding of outcome assessment (detection bias)
All studies were rated as having low risk for their detection bias since the assessors were blind to the maternal allocation.

3.5.16.5. Incomplete outcome data (attrition bias)
The study by Greenough et al. (2010) was rated as high risk since there was a high rate of loss to follow-up due to no response/drop outs. Attrition bias was assessed as low risk in the other included trials as there were an equal number of missing values reported in both arms of the studies.
3.5.16.6. Selective outcome reporting (reporting bias)
The pre-specified list of outcomes, in the published protocol and/or manuscript of the studies, have been reported; therefore all were rated as low risk.

3.5.16.7. Other potential sources of bias
The trial conducted by Greenough et al. (2010) was rated as unclear since the extended follow-up for this study was unplanned and there was an uncertainty as to whether participants were made aware of their allocation, and this could have disproportionately influenced which mothers decided to participate in the follow-up. All other studies were rated as low risk of bias.

3.5.17. Meta-analyses of effectiveness of maternal vitamin consumption during pregnancy and prevention of allergic diseases in the offspring
Pooled results from meta-analysis in the studies that examined the effectiveness of vitamin supplementation during pregnancy for prevention of allergic outcomes in offspring are presented in the following section. The allergic outcomes of wheeze, asthma, eczema and sIgE were reported in all the trials and were included in meta-analyses. We conducted sub-group meta-analyses for this dietary group since this is the first systematic review of RCTs investigating the effectiveness of prenatal intake of vitamins on allergic outcomes in children. The sub-group meta-analyses were conducted for the type of vitamins and control group. The study by Goldring et al. (2013) was included in sub-group meta-analysis, since it defined a different comparator with that of the other included trials i.e. no treatment. In addition, when interpreting the Forest plots, it is worth noting that the only available data for the Chawes et al. (2016) and Litonjua et al. (2016) studies was based on ITT analysis; however no imputation was performed for missing data in these studies.

3.5.17.1. Wheeze as the outcome measure for vitamin intervention
Four included studies measured the effect of vitamin consumption during pregnancy for prevention of wheeze in the offspring (Figure 3.27). The outcome of “recurrent or persistent wheeze” included in meta-analysis and was defined differently in the included studies. These are described as below:

**Greenough et al. (2010):** The outcome considered was “Wheeze, more than once a week” and was defined by asking women whether (or not) their infant had coughed and/or wheezed and the frequency of the cough and wheeze. The outcome was
reported as cumulative prevalence at 2 years of age.

**McEvoy et al. (2014):** The outcome considered was “medication for wheezing” and was defined by respiratory questionnaire (pediatric version) with the infant’s primary caretaker asking presence or absence of wheezing, medication for wheezing, maternal smoking, and exposure to second-hand smoke. The outcome was reported as the cumulative prevalence through age 1 year.

**Chawes et al. (2016):** The outcome considered was “Persistent wheeze” and was diagnosed according to a previously validated quantitative algorithm, requiring all of the following: i) recurrent wheeze (verified diary recordings of ≥5 episodes of troublesome lung symptoms [cough, wheeze, and/or dyspnea] lasting ≥3 days within 6 months), ii) typical symptoms of asthma (e.g. exercise-induced symptoms, prolonged nocturnal cough, or persistent cough outside common cold), iii) need for intermittent bronchodilator, and iv) response to a 3-month trial of inhaled corticosteroids and relapse upon cessation. The outcome was reported as the cumulative prevalence at 3 years.

**Litonjua et al. (2016):** The outcome considered was “Asthma or recurrent wheeze” and was defined by parental report of physician's diagnosis of asthma taken directly from the offspring questionnaires that was administered every three months. Recurrent wheeze was defined by the occurrence of at least one of the following five conditions: i) parental report of wheeze after child's second birthday preceded by at least 1 report of wheeze prior to second birthday; ii) report of child's use of asthma controller medication (defined as report of use of steroid inhalers or nebulizers, leukotriene modifiers, or steroid pills or liquids) after the second birthday, preceded by a report of wheeze before the second birthday; iii) 2 or more distinct parental reports of wheeze after the second birthday; iv) at least 1 parental report of wheeze and use of asthma controller medications at distinct visits, both subsequent to the second birthday; or v) 2 distinct reports of use of asthma controller medications after the second birthday. The outcome was reported as the cumulative prevalence at 3 years.

Statistically, there was no heterogeneity between the included studies ($\chi^2=0.73$, $P=0.86$, $I^2=0\%$). The result of meta-analysis demonstrated an association between vitamin consumption during pregnancy and the risk of developing wheeze in offspring (RR=0.79, 95% CI=0.66-0.95, 2,275 children).
3.5.17.2. Asthma as the outcome measure for vitamin intervention

In total two studies were included in a meta-analysis measuring the effect of vitamin intake during pregnancy for prevention of asthma in the offspring (Figure 3.28). The definitions of asthma, as described in the included trials, were as follows:

**Greenough et al (2010):** The outcome considered was “asthma, chest symptoms” (undefined) and was reported as cumulative prevalence in the first 12 months.

**Chawes et al. (2016):** The outcome considered was “Asthma” and was diagnosed in children, as secondary endpoint fulfilling the persistent wheeze criteria. The outcome was reported as point prevalence at 3 years.

There was a small statistical heterogeneity between the studies ($\chi^2=1$, $P=0.31$, $I^2=0.29\%$). The pooled results did show an association between maternal vitamin consumption during pregnancy and the risk of developing asthma in offspring (RR=0.75, 95% CI=0.53-1.05, 1,278 children).

**Figure 3.28. Forest plot of vitamins vs. placebo for asthma**

Measure: Binary; risk ratio
Heterogeneity: Q = 1; df = 1; p = 0.319; I-squared = 0.29%; tau-squared = 0.00184.
Random effects model: 0.754 (0.539, 1.05)
3.5.17.3. Eczema as the outcome measure for vitamin intervention

Three studies were included in a meta-analysis measuring the effect of vitamin intake during pregnancy for prevention of eczema in offspring (Figure 3.29). In the studies included in meta-analysis, eczema prevalence was defined as follows:

**Greenough et al. (2010):** The outcome considered was “Eczema” (undefined). The outcome was reported as cumulative prevalence in the first 12 months.

**Chawes et al. (2016):** The outcome considered was “Eczema” and was diagnosed according to the criteria of Hanifin including typical morphology and localisation of skin lesions. The outcome was reported as cumulative prevalence at 3 years.

**Litonjua et al. (2016):** The outcome considered was “Eczema with rash” and was defined by parental report of physician’s diagnosis of eczema with rash in typical distribution. The outcome was reported as cumulative prevalence at 3 years.

There was no heterogeneity between the included studies ($\chi^2=0.98$, $P=0.61$, $I^2=0\%$). The results of meta-analysis did not show an association between maternal vitamin consumption during pregnancy and the risk of developing eczema in offspring (RR=0.97, 95% CI=0.83-1.14, 2,139 children).

**Figure 3.29. Forest plot of vitamins vs. placebo for eczema**

Measure: Binary: risk ratio

Heterogeneity: Q = 0.982; df = 2; $p = 0.612$; I-squared = 0%; tau-squared = 0.

Random effects model: 0.977 (0.837, 1.14)
3.5.17.4. Raised specific Immunoglobulin E (sIgE) as an outcome measure for vitamin intervention

Three studies were included in a meta-analysis measuring the effect of vitamin intake during pregnancy raised level of sIgE in offspring (Figure 3.30). The definition of a positive sIgE result described in the included studies were as below:

**Chawes et al. (2016):** The outcome considered was “sIgE” and was defined by allergic sensitisation at 6 and 18 months by sIgE level of 0.35 kUA/L or higher against raw milk, pasteurized eggs, dogs, or cats. The outcome was reported as cumulative prevalence at 3 years.

**Litonjua et al. (2016):** The outcome considered was “Positive sIgE tests” and was defined as allergen sensitisation (specific IgE to a panel of aeroallergens and food allergens). The outcome was reported as point prevalence at 3 years.

A small level of heterogeneity was observed between the included studies ($\chi^2=1.01$, $P=0.31$, $I^2=0.98\%$). The results of meta-analysis did not show an association between maternal vitamin consumption during pregnancy and a positive sIgE in offspring (RR=0.95, 95% CI=0.68-1.31, 1,373 children).

**Figure 3.30. Forest plot of vitamins vs. placebo for positive sIgE**

Measure: Binary: risk ratio

Heterogeneity: $Q = 1.01; \ df = 1; \ p = 0.317; \ I\text{-squared} = 0.98\%; \ tau\text{-squared} = 0.000622.$

Random effects model: 0.95 (0.688, 1.31)
We also evaluated the efficacy of vitamin intake during pregnancy for prevention of allergic diseases for sub-groups according to “type of vitamin” and “type of comparison”.

3.5.17.5. Recurrent/persistent wheeze as the outcome measure in Vitamin D studies only

Sub-group analyses for the type of vitamin only included trials that used Vitamin D during pregnancy, since there was only one study for other types of vitamins. Sub-group analysis for studies that used “Vitamin D and applied placebo as the comparator” is shown in Figure 3.31. The trials were statistically homogenous ($\chi^2=0.002, P=0.95, I^2=0\%$). The results of meta-analysis showed an association between prenatal intake of Vitamin D and the risk of developing persistent wheeze in offspring (RR=0.80, 95% CI=0.66-0.97, 1,387 children).

Figure 3.31. Forest plot of Vitamin D vs. placebo for recurrent/persistent wheeze

Measure: Binary: risk ratio
Heterogeneity: Q = 0.00287; df = 1; p = 0.957; I-squared = 0%; tau-squared = 0.
Random effects model: 0.806 (0.666, 0.976)
3.5.17.6. Recurrent/persistent wheeze as the outcome measure in Vitamin D studies versus placebo or no treatment

Three trials that used Vitamin D and applied either “placebo or no treatment as the comparator” were included in a sub-group analysis (Figure 3.32). The study by Greenough conducted two separate analyses for combined Vitamin D (Bolus + daily) and daily intake and therefore, these were included separately in meta-analyses.

The outcome considered for Goldring et al. (2013) study was “Recurrent wheezing (≥2 episodes of reported wheezing since birth), defined by ISAAC criteria”. The outcome was reported as point prevalence at 3 years. No statistical heterogeneity was observed between the included trials ($\chi^2=0.61$, $P=0.73$, $I^2=0\%$) and a beneficial association was shown between prenatal intake of Vitamin D and risk of developing persistent wheeze in the offspring (RR=0.82, 95% CI=0.68-0.988, 1,545 children).

Figure 3.32. Forest plot of daily and/or combined Vitamin D vs. placebo or no treatment as the control for recurrent/persistent wheeze

Measure: Binary: risk ratio
Heterogeneity: $Q = 0.613; df = 2; p = 0.736; I$-squared = 0%; $tau$-squared = 0.
Random effects model: 0.82 (0.681, 0.988)

<table>
<thead>
<tr>
<th>Outcome: Wheeze-Daily/combined Vitamin D versus placebo &amp; no treatment</th>
<th>Vitamin D n/N</th>
<th>Control n/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goldring 2013</td>
<td>17/108</td>
<td>7/50</td>
</tr>
<tr>
<td>Chawes 2016</td>
<td>47/295</td>
<td>57/286</td>
</tr>
<tr>
<td>Litonjua 2016</td>
<td>98/405</td>
<td>120/401</td>
</tr>
<tr>
<td>Subtotal</td>
<td>808</td>
<td>737</td>
</tr>
</tbody>
</table>

The meta-analysis for intake of daily vitamin D only as opposed to either placebo or no treatment (Figure 3.33) also showed a protective effect between prenatal intake of vitamins and the risk of developing persistent wheeze in offspring (RR=0.812, 95% CI=0.673-0.98, 1,493 children).
Figure 3.33. Forest plot of daily Vitamin D vs. placebo or no treatment as the control for recurrent/persistent wheeze

Measure: Binary: risk ratio  
Heterogeneity: Q = 0.163; df = 2; p = 0.922; I-squared = 0%; tau-squared = 0  
Random effects model: 0.812 (0.673, 0.98)

<table>
<thead>
<tr>
<th>Outcome: Wheeze-Daily Vitamin D versus placebo or no treatment</th>
<th>Vitamin D n/N</th>
<th>Control n/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goldring 2013</td>
<td>7/50</td>
<td>8/56</td>
</tr>
<tr>
<td>Chawes 2016</td>
<td>47/295</td>
<td>57/286</td>
</tr>
<tr>
<td>Litonjua 2016</td>
<td>98/405</td>
<td>120/401</td>
</tr>
<tr>
<td><strong>Subtotal</strong></td>
<td><strong>750</strong></td>
<td><strong>743</strong></td>
</tr>
</tbody>
</table>

3.5.18. Discussion of the evidence synthesis of vitamin consumption during pregnancy and prevention of allergic diseases in the offspring

3.5.18.1 Summary of main results

This systematic review summarised data from five RCTs of maternal intake of vitamins for prevention of childhood allergic diseases, including a total of 2,456 children with follow-up duration ranging from 1-3 years (mean 2.4 years). The studies were at risk of bias with 40% rated as unclear and a high risk of bias for allocation concealment and performance respectively. Studies were also varied for the type and dose of vitamin supplemented, duration of follow-up and their sample size. The findings from this systematic review and meta-analysis do not provide evidence of a protective association between intake of vitamins during pregnancy and subsequent development of a number of allergic manifestations including asthma, eczema and sensitisation to allergens as measured by sIgE. However, there appeared to be an effect for recurrent wheeze. The meta-analysis of four studies showed a significantly reduced risk of developing recurrent wheeze in offspring (RR=0.79, 95% CI=0.66-0.95, 2,275 children). These results need to be interpreted with caution because of the risk of bias, heterogeneity observed between studies as well as small number of studies that contributed to the meta-analyses.
3.5.18.2. Overall completeness and applicability of evidence
There was a low level of evidence that vitamin supplementation during pregnancy is effective for prevention of a number of allergic outcomes in the offspring. The meta-analyses for “recurrent wheeze” were also limited by the number of studies included and also a small sample size in most trials. The heterogeneity between the trials limited the findings and random effect models were used to pool the results. Heterogeneity between studies originated from varied types and dose of vitamins, different timing and duration of interventions, as well as the follow-up duration and a sample size. Trials also used different methods for measuring and reporting allergic outcomes.

3.5.18.3. Quality of evidence
Overall, the trials were at moderate to high risk of bias, for the summary of risk of bias assessment (Figure 3. 26). Some studies were rated as unclear or high risk of bias for individual quality domains. Un-blinded detection bias and high loss to follow-up were judged in one study in each domain (20%). Also, the small number of studies and participants that contributed to the meta-analyses might have downgraded the quality of body of evidence.

3.5.18.4. Strength and weakness of this systematic review for vitamin consumption during pregnancy
The results from this unique systematic review provided an overview of the current body of literature on the effectiveness of prenatal intake of vitamins and the risk of developing allergic diseases in the offspring. Following an a priori published protocol, we used a comprehensive search strategy that allowed for a complete cover of all the relevant literature through citation databases, trial registries and conference proceedings, identifying five RCTs with a total of 2,456 children. Furthermore, we were interested in any reported allergic outcomes from these trials; however a limited number of allergic diseases were reported in included trials, mostly reporting the pulmonary function outcomes.

The limitation of our systematic review stems from the limitations of the included trials. We were unable to perform any meta-analyses on the timing or dose of intervention and study populations due to a relatively small number of trials that could contribute.
3.5.18.5. Agreement and disagreement with other systematic reviews or studies
This is the first systematic review and meta-analysis of RCTs that investigated the association between prenatal intake of vitamins and the risk of developing allergic diseases in the offspring. The results however, are in line with observational studies that have typically reported a beneficial effect of higher intake of Vitamin D during pregnancy on allergic outcomes (Allan, Kelly, & Devereux, 2010; Nurmatov, Devereux, & Sheikh, 2011; Palmer, Sullivan, Skeaff, Smithers, & Makrides, 2015).

3.5.18.6. Author’s conclusion

Implications for practice
The current body of evidence suggests that there is an association between prenatal supplementation of Vitamin D and prevention of recurrent/persistent wheezing in children. However the certainty of evidence is low due to the number of studies and discrepancies between studies. There is a lack of evidence for the effect of prenatal intake of vitamins and developing other allergic outcomes.

Implications for research
Taking the quantity and quality of the available evidence into account, the effect of vitamins intake during pregnancy for prevention of childhood allergies needs to be further investigated in large well-designed and executed RCTs.

Timing of intervention is a key factor that needs to be further investigated. In all trials the intervention was started in the second trimester in pregnancy. However, the development of the lungs begins in the first trimester in pregnancy and Vitamin D plays an immunomodulatory role in the development of the lung and immune system (Henderson & Warner, 2012). Therefore the interventions might have commenced too late in pregnancy, or used too low dose of Vitamin D to have a beneficial impact on lung development. Furthermore, the studies recruited different types of population, which limits the generalisability of the studies. Baseline levels of Vitamin D vary in different geographical areas (Kimlin, Olds, & Moore, 2007), and this issue has not been addressed in the conducted trials. Well-designed trials are necessary to address all these possible confounders among different populations (Karras et al., 2015).
It is also possible that the follow-up periods of the studies for this review have been too short to detect other allergic outcomes. For example, wheezing is known as a primary symptom of asthma in early childhood (Stevens, Turner, Kuehni, Couriel, & Silverman, 2003). About 40% of childhood wheeze will persist later in life, and will eventually develop into asthma by 6 years of age (Martinez et al., 1995), indicating that the majority of wheeze during infancy is in fact acute respiratory infection. Therefore, extended follow-up of these trials could help to provide a clearer answer as to whether the Vitamin D intervention is beneficial for asthma prevention.

There were also some limitations in the design of studies. For example, the trials were statistically underpowered to detect an effect for their primary and/or secondary outcome measures. Significant differences were only observed for some of the secondary outcomes as “at least 1 episode of wheezing” (McEvoy et al., 2014), “episodes of troublesome lung symptoms” (Chawes et al., 2016) and “positive sIgE” (Litonjua et al., 2016) and trials failed to show a beneficial effect for primary allergic outcomes such as wheeze and asthma in children. Also, the trials used different doses of vitamins during pregnancy. The dose of Vitamin D varied between 800-4000IU and doses of Vitamin C and/or E, varied between 500-1000mg. It is possible that lower doses of vitamins may have failed to reach the desirable level of 25-hydroxyvitamin D or antioxidants in pregnant women to have an influential effect on the foetal immune programming and lung function (Holick, 2007; Litonjua & Weiss, 2007; Yurt et al., 2014). However this is refuted by studies which have reported a similar size using higher doses of Vitamin D (Chawes et al., 2016; Litonjua et al., 2016). Safety and efficacy of Vitamin D supplementation during pregnancy is addressed in a RCT and it showed that a 4000IU dose of Vitamin D was a safe approach and is necessary to optimise the circulating concentration of 25-hydroxy Vitamin D levels to ≥ 80nmol/L (Hollis, Johnson, Hulsey, Ebeling, & Wagner, 2011). There is limited evidence on the safety of Vitamins C and E intake at any stage of pregnancy; however the Institute of Medicine’s Food and Nutrition Board have set an upper limit of 2000mg and 1000mg per day for Vitamins C and E ingestion respectively during pregnancy in the United States (Institute of Medicine, 2000).

Moreover, the role of maternal consumption of vitamins during pregnancy on the risk of developing other allergic outcomes and sensitisation needs to be investigated in
larger well-designed trials. Human in vitro studies suggest that antioxidants e.g. Vitamins A, C, D and E can improve immune function in human (Utsugi et al., 2003).

3.6. Round-up conclusion of the chapter

The current body of evidence in terms of quantity as well as quality of the conducted RCTs makes the strength of the current findings fairly unreliable and underscores the importance of prenatal intake of dietary interventions for prevention of most allergic outcomes in the offspring. Large well-designed and well executed nutritional intervention studies that could target pregnant women in pre-pregnancy and as early as in pregnancy could provide a better understanding of the role of nutrition immune programming and its role for the prevention of childhood allergic disorders. It is advised that nutritional trials ought to consider clearly defined rules and objectives in their design such as measurement of basal nutrient status and the change following the intake of intervention, enabling to validly test the hypothesised association(s) (Heaney, 2014).
Chapter 4: The effectiveness of maternal nutritional/dietary interventions during pregnancy and risk of developing obesity in the offspring: systematic reviews and meta-analyses

4.1. Overview of the chapter

This chapter presents systematic reviews of literature describing the most recent available evidence from randomised controlled trials on the effectiveness of maternal dietary interventions during pregnancy for the prevention of obesity in offspring. Dietary interventions explored include fatty acids, pro/prebiotics, low glycemic index, lifestyle change and vitamins/supplements. The methodology section outlines: how the comprehensive literature search was conducted, the eligibility criteria used to assess studies against the defined inclusion criteria, and the techniques used for assessing the quality of included studies. The results are sectioned according to the intervention type and risk of bias (ROB) assessment. Where possible, the effect of outcomes have been presented using meta-analyses and where not possible, through narrative description. For each intervention type, discussion sections summarise the findings from this systematic review, making comparisons with the current literature and defining areas for further research.

4.2. Objectives

To conduct systematic reviews assessing the effectiveness of maternal dietary interventions during pregnancy on the prevention of obesity in offspring

4.3. Methods and protocol for the systematic reviews

With the exception of the included outcomes, the protocol developed for assessing the efficacy of prenatal dietary interventions during pregnancy on allergic outcomes in offspring was utilised for this systematic review (appendix 3.1). Hence, the methods for conducting this systematic review have already been described in Chapter 3, but where appropriate, additional details are described below.
4.3.1. Criteria for considering studies for review

4.3.1.1. Types of studies
Please refer to section 3.3.1.1 in Chapter 3

4.3.1.2. Types of participants
Please refer to section 3.3.1.2 in Chapter 3

4.3.1.3. Types of interventions
Studies reporting one or more of the following interventions during pregnancy were included:

a. Fatty acid supplementations
b. Pro/prebiotic supplementations
c. Food-based dietary advice (promoting a healthy diet) alone or in combination with supervised exercise/physical activity i.e. low glycemic (LG) index diet, lifestyle change (nutritional counselling)
d. Vitamin/multivitamin, micronutrients and minerals (will be mentioned as vitamins hereafter)

Trials were also included if the intervention(s) had been extended after pregnancy either in breast-feeding mothers, the infants or both. Studies within each of the above mentioned interventions were grouped under a common type of intervention i.e. any fatty acids, pro/prebiotics, LG index diet, life-style change or vitamins, regardless of their specific applied intervention.

4.3.1.4. Outcomes of interest
Trials were included if they had reported obesity as an outcome, either as a primary or secondary endpoint, in the offspring from infancy to adulthood. The primary and secondary outcome measures were defined by body composition outcomes.

4.3.2. Search strategy for identification of studies
A comprehensive search strategy, including all the relevant synonyms for the main concepts, was developed covering the main bibliographic databases (see appendix 4.1).

4.3.2.1. Electronic searches
Please refer to section 3.3.2.1 in Chapter 3
4.3.2.2. Searching other sources of evidence

References of all identified studies and key systematic reviews in this area were checked for potentially relevant studies not identified by the above search strategy. To date, there are five systematic reviews that have evaluated the use of prenatal and/or postnatal fatty acids for the prevention of obesity in offspring. Three systematic reviews did not perform meta-analyses (Campoy, Escolano-Margarit, Anjos, Szajewska, & Uauy, 2012; Muhlhauser, Gibson, & Makrides, 2010; Rodriguez, Iglesia, Bel-Serrat, & Moreno, 2012) and narratively described the effectiveness of prenatal and/or postnatal supplementation with n-3 LCPUFA on an infant’s body composition. Another systematic review (Imhoff-Kunsch, Briggs, Goldenberg, & Ramakrishnan, 2012) included RCTs that used prenatal n-3 LCPUFA, in high and low risk pregnancies, and investigated the influence of the intervention on maternal, neonatal and child health at birth only. The most recent systematic review by Stratakis (Stratakis, Gielen, Chatzi, & Zeegers, 2014) included both prenatal and postnatal RCTs that used n-3 LCPUFA and conducted meta-analyses and sub-group analyses only for BMI. In the present systematic review, we included RCTs that involved only prenatal intake of fatty acids (as well as those in which intake continued postnatally) and considered a range of the reported outcomes on obesity.

There are also systematic reviews that evaluated the effectiveness of ‘lifestyle interventions’ or ‘dietary counselling in combination with physical activity’ during pregnancy, considering only maternal outcomes such as gestational weight gain and obstetrics outcomes e.g. birth weight, small for gestational age (SGA) or large for gestational age (LGA) (Campbell, Johnson, Messina, Guillaume, & Goyder, 2011; Dodd, Grivell, Crowther, & Robinson, 2010; Gardner, Wardle, Poston, & Croker, 2011; Muktabhant, Lawrie, Lumbiganon, & Laopaiboon, 2015; Ronnberg & Nilsson, 2010; Skouteris et al., 2010; Streuling, Beyerlein, & von Kries, 2010; Sui, Grivell, & Dodd, 2012; Tanentsapf, Heitmann, & Adegboye, 2011; Thangaratinam et al., 2012) No systematic reviews to date investigated the longer-term efficacy of life-style interventions for prevention of childhood obesity. When this review was conducted, there were also no published systematic reviews for prevention of childhood obesity for probiotics, LG index diet and vitamin interventions.
The uniqueness of the present systematic review is therefore that it aims to explore a variety of the dietary interventions that have only commenced during pregnancy, and to investigate the effectiveness of these interventions for prevention of a range of reported obesity outcomes in the offspring.

4.3.3. Data collection and analysis

4.3.3.1. Selection of studies

Please refer to section 3.3.3.1 in Chapter 3. The relevant appendices are 4.2 and 4.3.

4.3.3.2. Data extraction and management

Please refer to section 3.3.3.2 in Chapter 3. Where relevant, changes were made in the data extraction such as type of studied sample, detailed information about type of intervention (fatty acids, probiotics, LG index, lifestyle change and vitamins) and the reported outcomes (appendix 4.4).

4.3.4. Assessment of risk of bias in included studies

Please refer to section 3.3.4 in Chapter 3.

4.3.4.1. Measurement of treatment effect

Please refer to section 3.3.4.1 in Chapter 3.

4.3.4.2. Unit of analysis issues

Please refer to section 3.3.4.2 in Chapter 3.

4.3.4.3. Handling missing data

Please refer to section 3.3.4.3 in Chapter 3. Studies that performed ITT analysis are also identified in the table of characteristics of included studies (Table 4.2).

4.3.4.4. Assessment of heterogeneity

To measure statistical heterogeneity between effect sizes of included studies, within each umbrella intervention group and for each obesity outcome separately, we used visual inspection of forest plots and also the $\chi^2$ test for heterogeneity with a P Value <0.05 (Deeks, et al., 2001). $I^2$ statistics were used to quantify the amount of possible variability in effect estimates that is due to heterogeneity rather than chance ($I^2 >30\%$ moderate heterogeneity, $I^2 \geq 75\%$ considerable heterogeneity). Moreover, studies with
a similar comparator within each umbrella intervention group were grouped to run meta-analyses.

With regards to clinical heterogeneity, two key issues were considered. Firstly, the crude data for any reported obesity outcomes from the included trials were extracted. The following outcomes were considered for this systematic review (Table 4.1).

Table 4.1. List of the outcomes of interest for this systematic review

<table>
<thead>
<tr>
<th>Main Obesity outcomes</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Mass Index (BMI)</td>
<td>Latest available follow-up data</td>
</tr>
<tr>
<td>BMI-Z score</td>
<td>Latest available follow-up data</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>Latest available follow-up data</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>Latest available follow-up data</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>Latest available follow-up data</td>
</tr>
<tr>
<td>Skin Fold Thickness (SFT)</td>
<td>Latest available follow-up data</td>
</tr>
<tr>
<td>Obesity</td>
<td>Latest available follow-up data</td>
</tr>
<tr>
<td>Overweight</td>
<td>Latest available follow-up data</td>
</tr>
</tbody>
</table>

Secondly, the longest follow-up measures, where available, were selected for this systematic review and entered in the meta-analyses. Moreover, where reported, cumulative prevalence/incidence were included in meta-analyses and if not reported, point prevalence(s) were considered. Sub-group meta-analyses were conducted where there were an adequate number of studies that could contribute in meta-analysis for the following criteria:

a. Type of the comparator
b. Length of follow-up

4.3.4.5. Assessment of reporting biases

Every effort was made to identify unpublished studies through searching abstracts and ongoing trials databases as described in section 3.3.2. Publication bias was not evaluated since the number of studies within each intervention group were less than 10.

4.3.4.6. Data synthesis

This section compares with the 3.3.4.6 section in Chapter 3, with the exception of one minor change regarding the nature of extracted data. Continuous data were entered as means and standard deviations.
4.4. Changes to the protocol

There were five changes made to the protocol during the conduct of the review. Firstly, we stated in the protocol that studies with a maximum follow-up duration of 18 years will be included. However, there was an exception to this in that we included a study with a follow-up period of 19 years in the current systematic review, and the effect of duration of follow-up on obesity outcomes in children was assessed in sub-group analyses.

Secondly, in the protocol for this review, it was initially stated that in trials with multiple intervention groups, data for the control group would be used for each intervention group comparison and the weight assigned to the control group will be reduced by dividing the number of participants in the control group by the number of intervention groups. However, for more clarity, and as recommended by the Cochrane Collaboration (Higgins et al., 2011), it was decided to pool the data for each intervention arm for different probiotic strains only, and make the comparison for the pooled probiotic intervention arm versus the control group. So, the weight assigned to the intervention group was considered as the total number of participants in intervention arms divided by the number of participants in the control group. It is important to add that we did not pool the data for different intervention arms in fatty acids studies versus the control group, since the individual intervention arms were entirely different in their nature. Therefore only the data for the fatty acids (fish oil) intervention arm was considered as opposed to the control group for this systematic review.

Thirdly, there were some cases for which we included the data on an earlier available follow-up point in meta-analyses since this could allow a more consistent outcome measure assessment across a number of the included studies. For example, where there were outcomes reported at 7 and 10 year follow-up points in a study that used probiotics as an intervention, the 7 years follow-up data was included in meta-analyses since it was closer to the follow-up points in the other included studies.

Additionally, due to the discrepancies observed between the included studies (e.g. differences in primary and secondary endpoints, different comparators) as well as the
small number of studies that met the inclusion criteria in the dietary groups, it was
decided not to perform detailed sub-group meta-analyses for the duration of
intervention, specific type of consumed dietary products and
dosage/frequency/delivery mode of intervention.

Lastly, it was intended to use the per-protocol analysis for conducting all the meta-
analyses; however this was not possible for a few papers as the ITT was the only
available data. In these cases the reported data were used in performing meta-
analyses.

4.5. Results

4.5.1. Studies identified through searches and total included studies
Searches of electronic databases were initially carried out between November and
December 2014 and updated by the end of January 2016. The searches yielded a total
of 10,127 references. As specified in the protocol, removal of duplicates and non-
relevant studies (6,834) from the 6,898 included studies for screening the title and
abstract left 65 papers for further consideration. Of the remaining 65 references, 22
were excluded after closer inspection showed that they had either reported an
inappropriate study outcome or had inappropriate study design/participants.

Full-text screening of the remaining 43 papers showed that they were either a study
protocol (n=4) or earlier published reports of the included studies (n=21). These
earlier reports, hereafter referred to as ‘linked records/companion papers’, were used
to extract any relevant data of the initial trial, if required. Supplementary searches
from other databases identified one extra paper. As a result, a total number of 19
studies were included in this systematic review. The PRISMA flow diagram is shown
in Figure 4.1.
Figure 4.1. PRISMA flowchart for the literature review strategy-Obesity outcomes

The list of the included studies that met the inclusion criteria for this systematic review, by intervention type, is shown in Table 4.2. All trials were in English and had been carried out in various countries across the world. The descriptive findings, risk of bias assessment and effects of interventions are structured by intervention in the following sections.
It is important to note that studies which used vitamin/micronutrients, including one study using protein-energy supplements, were conducted in developing countries aiming to boost the growth standards of infants at birth. These studies met the inclusion criteria for this systematic review and were therefore included in the review; however, their results are only described narratively in a separate section and no meta-analyses were conducted for this intervention group.

Table 4.2. List of the included studies

<table>
<thead>
<tr>
<th>Fatty Acids Interventions</th>
<th>AOD</th>
<th>ITT</th>
<th>LG index diet Interventions</th>
<th>AOD</th>
<th>ITT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Helland 2008</td>
<td>✓</td>
<td>NI</td>
<td>Louie 2015</td>
<td>✓</td>
<td>NI</td>
</tr>
<tr>
<td>Dunstan 2008</td>
<td>✓</td>
<td>NI</td>
<td>Horan 2016</td>
<td>✓</td>
<td>NI</td>
</tr>
<tr>
<td>Campoy 2011</td>
<td>✓</td>
<td>NI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rytter 2011</td>
<td>✓</td>
<td>NI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stein 2015</td>
<td>✓</td>
<td>NI</td>
<td>Tanvig 2015</td>
<td>✓</td>
<td>NI</td>
</tr>
<tr>
<td>Bergmann 2014</td>
<td>-</td>
<td>✓</td>
<td>Rauh 2015</td>
<td>✓</td>
<td>NI</td>
</tr>
<tr>
<td>Brei 2016</td>
<td>✓</td>
<td>NI</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Life-style change Interventions</th>
<th>AOD</th>
<th>ITT</th>
<th>LG index diet Interventions</th>
<th>AOD</th>
<th>ITT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>✓</td>
<td></td>
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<tr>
<td></td>
<td>✓</td>
<td></td>
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</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Probiotics Interventions</th>
<th>AOD</th>
<th>ITT</th>
<th>LG index diet Interventions</th>
<th>AOD</th>
<th>ITT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kuitunen 2009</td>
<td>✓</td>
<td>NI</td>
<td>Roth 2013</td>
<td>✓</td>
<td>NI</td>
</tr>
<tr>
<td>Luoto 2010</td>
<td>✓</td>
<td>-</td>
<td>Stewart 2009</td>
<td>✓</td>
<td>NI</td>
</tr>
<tr>
<td>Abrahamsson 2013</td>
<td>✓</td>
<td>NI</td>
<td>Vaidya (2008)</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Wickens 2013</td>
<td>✓</td>
<td>✓</td>
<td>Halksworth 2008</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>

*For studies with data available on both observed and ITT analysis, the observed data are included in meta-analysis. Ante. AOD=Analysis on the Observed Data; ITT=Intention to Treat Analysis, some trials have conducted the ITT only for certain outcomes; NI=No information

4.5.2. Presentation of the results

The meta-analyses for assessing the effect of intervention are structured by intervention type. Within each intervention category, studies are grouped and assessed for the clinical outcome(s) of interest for this systematic review. Detailed descriptions of the outcomes included in the meta-analyses are also presented. As described in section 4.3.4.4, meta-analyses were conducted using a random effect model and where relevant, sub group analyses have been conducted in different dietary intervention groups.

4.5.3. Description of included studies of maternal fatty acid consumption during pregnancy and prevention of obesity in the offspring

Of the 19 included trials, 7 (with a total of 1,647 children) examined the impact of fatty acid interventions on the development of obesity in offspring. The detailed
characteristics of the included trials, their companion papers and study population are shown in Table 4.3. One study was multi-centre in Germany, Spain and Hungary; two studies were conducted in Germany and the rest in Australia, Norway, Denmark and Mexico.

It is worth noting that most of the trials were primarily designed to investigate other outcomes in children such as neurological development, and using post-hoc analyses, they also reported growth measures in the offspring (Bergmann et al., 2012; Campoy et al., 2011; Dunstan, Simmer, Dixon, & Prescott, 2008; Gonzalez-Casanova et al., 2015; Helland et al., 2008; Rytter et al., 2011). The study by Brei (Brei et al., 2016) was the first trial designed to evaluate the effectiveness of prenatal n-3 LCPUFA supplementation on infant’s body composition.

The longest follow-up period was 19 years, in the study conducted by Rytter et al. (2011), followed by 7 years follow-up in the study by Helland et al. (2008). The shortest follow-up period was 2.5 years, in the study conducted by Dunstan et al. (2008). The largest study sample was reported in Gonzalez-Casanova et al. (2015) with 1,094 mothers enrolled followed by Rytter et al. (2011) involving 533 pregnant mothers. The smallest sample sizes were found in studies conducted by Dunstan et al. (2008) and Bergman et al. (2012) with 98 and 144 mothers randomised at recruitment respectively.

With the exception of the study conducted by Dunstan and colleagues (2008) that enrolled atopic women in their trial, the remainder recruited healthy pregnant women with non-complicated pregnancies and most frequently reported outcomes were BMI, weight, and height.

Compliance with the intervention was assessed by a variety of methods, including the number of fatty acids capsules ingested, divided by the number the participant should have ingested multiplied by 100, standardised questionnaires at gestation, the percentage of the total number of capsules expected to be consumed and measuring fatty acids levels in erythrocytes at 30 and 37 gestational week and 6 weeks postnatally. Two studies did not report the method of measuring adherence to the intervention (Bergmann et al., 2012; Helland et al., 2008).
Table 4.3. Characteristics of the included trials and study population of fatty acids and prevention of obesity

<table>
<thead>
<tr>
<th>Primary article</th>
<th>Companion articles</th>
<th>Country, enrolment period</th>
<th>Trial type</th>
<th>Study intervention &amp; comparator</th>
<th>No. of participants **</th>
<th>No. at last F-U***</th>
<th>Time points measured</th>
<th>Age at last F-U</th>
<th>Sample: high or low risk</th>
<th>Outcomes reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Helland et al., 2008)</td>
<td>(Helland et al., 2001)</td>
<td>Norway 1994-96</td>
<td>PC-RCT</td>
<td>N-3 FA &amp; control</td>
<td>Pregnancy: 590</td>
<td>143: 82 vs. 61</td>
<td>Birth, 6 &amp; 9 months, 4 &amp; 7yrs</td>
<td>7yrs.</td>
<td>Healthy women</td>
<td>-BMI (kg/m²) -Weight (Kg) -Height (cm)</td>
</tr>
<tr>
<td>(Dunstan, et al., 2008)</td>
<td>(Dunstan et al., 2003; Dunstan, et al., 2004)</td>
<td>Australia 2000-2001</td>
<td>PC-RCT</td>
<td>Fish oil &amp; olive oil</td>
<td>98 mothers</td>
<td>72: 33 vs. 39</td>
<td>Birth, 2.5yrs</td>
<td>2.5yrs.</td>
<td>Women with allergic disease</td>
<td>-Weight -Height</td>
</tr>
<tr>
<td>(Campoy et al., 2011)</td>
<td>(Decsi, Campoy, &amp; Koletzko, 2005; Escolano-margarit, Ramos, &amp; Beyer, 2011; Krauss-ETSCHMANN et al., 2007)</td>
<td>Germany Spain Hungary 2001-03</td>
<td>PC-RCT</td>
<td>Fish-oil vs. FO+5MTHF*a vs. 5-MTHF vs. Placebo</td>
<td>311 mothers</td>
<td>154: 37 vs. 35 vs. 45</td>
<td>Birth, 4 &amp; 6.5yrs</td>
<td>6.5yrs.</td>
<td>Healthy women</td>
<td>-BMI (kg/m²)</td>
</tr>
<tr>
<td>(Ryter et al., 2011)</td>
<td>(Olsen et al., 1992)</td>
<td>Denmark 1989-90</td>
<td>PC-RCT</td>
<td>Fish Oil vs. either Olive oil or No oil</td>
<td>533 mothers</td>
<td>243: 108 vs. 63 vs. 72</td>
<td>19yrs.</td>
<td>19yrs.</td>
<td>Non complicated pregnancies + no allergy to fish</td>
<td>-BMI (kg/m²) -Waist (cm) -Insulin (pmol/L) -Blood glucose (mmol/L) -Hb A1c fraction (%) -HOMA-IR -Leptin (Ig/L) -Adiponectin (microg/L) -IGF-I (microg/L) -hs-CRP (mg/L)</td>
</tr>
<tr>
<td>(Bergmann et al., 2012)</td>
<td>(Bergmann et al., 2007; Bergmann et al., 2008)</td>
<td>Germany, Berlin 2000-02</td>
<td>PC-RCT</td>
<td>Fish oil + Vit/Min + FOS vs. two controls</td>
<td>144 mothers</td>
<td>115: 41 vs. 74 (DHA vs. pooled control groups)</td>
<td>1, 3, 21 months &amp; 6yrs</td>
<td>6yrs.</td>
<td>Healthy pregnant Caucasian women</td>
<td>-Weight (Kg) -Height (cm) -BMI (kg/m²) -BMI Z-score -Sum 4 SFT [mm] -Head Circumference (cm)</td>
</tr>
<tr>
<td>(Gonzalez-</td>
<td>(Ramakrishnan et al., 2009)</td>
<td>Mexico</td>
<td>PC-</td>
<td>DHA &amp; placebo</td>
<td>1,094 mothers</td>
<td>802: 403</td>
<td>1, 3, 6, 9, 5yrs.</td>
<td>Non complicated</td>
<td>-BMI Z-score</td>
<td></td>
</tr>
<tr>
<td>Casanova et al., 2015</td>
<td>2010; Stein et al., 2011</td>
<td>2005-07</td>
<td>RCT</td>
<td>vs. 399</td>
<td>12,18months &amp; 5yrs.</td>
<td>pregnancies</td>
<td></td>
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</tr>
<tr>
<td>(Brei et al., 2016)†</td>
<td>(Hauner et al., 2012) (Brunner et al., 2013) (Much et al., 2013) (Brunner et al., 2015)</td>
<td>Germany, Munich 2006-09</td>
<td>RCT</td>
<td>Fish oil &amp; control</td>
<td>208 mothers</td>
<td>118: 61 vs. 57</td>
<td>4months, 1, 2 &amp; 5 years</td>
<td>5yrs.</td>
<td>Healthy women</td>
<td></td>
</tr>
</tbody>
</table>

| -Weight (Kg) | -Height (cm) | -Height for age z-score | -Weight for age z-score |

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1^Published data and conference presentations, no unique data were extracted from conference abstracts

2^Indicates the number at randomisation, where recruitment has occurred prenatally

3^Methyl tetra-hydro folic

4^Published on May 2016 and data for 5 years follow-up was obtained from personal communication with author, following the update of search strategy on January 2016

5^Placebo Controlled-Randomised Controlled Trial

6^Follow-up

7^Fructo-oligosaccharide

8^Fructo-oligosaccharide
Table 4.4 shows the details of the fatty acid interventions and placebo used in included trials. In all studies the intervention and placebo groups had comparable baseline characteristics at recruitment. It is worth noting that the Helland et al. (2008) study reported mean age of the mothers receiving cod liver oil was, by chance, 1 year higher than the age of the mothers receiving corn oil (p≤0.01).

Three studies administered fatty acids supplements solely during pregnancy (Dunstan et al., 2008; Gonzalez-Casanova et al., 2015; Rytter et al., 2011). Whereas in the remainder, fatty acid interventions were continued after pregnancy either in mothers only or in infants (Table 4.4). The starting time of intervention varied in the trials, beginning as early as 15 weeks of gestation (Brei et al., 2016) or as late as week 30 of gestation (Rytter et al., 2011) with the longest duration of intervention being 10-10.5 months (Campoy et al., 2011), and the shortest 2-2.5 months (Rytter et al., 2011).

With the exception of one study that only used algal DHA (Gonzalez-Casanova et al., 2015), all other studies used a combination of EPA and DHA together. In the study by Campoy et al., (2011) vitamin and mineral supplements were also used along with the fatty acid component. Furthermore, Brei et al., 2016 provided women with fatty acid intervention as well as nutritional counseling, focused on normalising the consumption of n-6 fatty acid (AA) to a moderate level of intake (90 mg AA per day).

The fatty acid preparations were delivered as capsules in four of the studies (Brei et al., 2016; Dunstan, et al., 2008; Gonzalez-Casanova et al., 2015; Rytter et al., 2011), with a further two using milk-based supplements (Bergmann et al., 2012; Campoy et al., 2011;) and another study providing oil preparations (Helland et al., 2008).

The choices of intervention in the Campoy et al. (2011) study were defined within three categories: modified fish oil plus vitamin and mineral, fish oil plus 5-methyltetrahydrofolic (MTHF) and 5-MTHF only. In this systematic review, we have only considered modified fish oil component plus vitamin and mineral as the intervention arm compared to placebo.

In addition, the diversity of comparators between studies were as follows: corn oil (Helland et al., 2008), a mixture of corn and soy oil (Gonzalez-Casanova et al., 2015),
olive oil (Dunstan et al., 2008; Rytter et al., 2011), basic vitamin and mineral (Bergmann et al., 2012), standard diet (Brei et al., 2016) and no information in one study (Campoy et al., 2011). The Bergmann et al. (2012) study originally defined either ‘basic vitamin plus mineral’ or ‘prebiotic FOS’ as the comparators and at 6 years follow-up, the authors presented the data for the combined control groups versus fatty acids component. The study by Rytter et al. (2011) also had the choice of either ‘olive oil’ or ‘no oil’ and for the purposes of this systematic review, we considered the fatty acids versus ‘olive oil’ in the meta-analysis.

The study by Brei et al. (2016) that introduced ‘standard diet’ as its comparator, was included in sub-group meta-analysis, since this was not similar in nature to the other comparators.
Table 4.4. Characteristics of fatty acid interventions in the included trials

<table>
<thead>
<tr>
<th>Primary article</th>
<th>Comparable baseline characteristic</th>
<th>Participants receiving intervention</th>
<th>Timing of Intervention in pregnancy</th>
<th>Intake of Intervention from/until</th>
<th>Duration of intervention (months)</th>
<th>Fatty acids product ***</th>
<th>Placebo/Control</th>
<th>Mode of delivery</th>
<th>Total daily dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Helland et al., 2008</td>
<td>No Mean age of the mothers receiving cod liver oil was, by chance, 1 year higher than the age of the mothers receiving corn oil</td>
<td>Prenatally &amp; postnatally-in mothers</td>
<td>From 18 wks.</td>
<td>18 wks. to 3 months postnatal</td>
<td>8-8.5</td>
<td>Cod liver oil (1183 mg/10 mL of DHA, 803 mg/mL of EPA; 20:5N-3) &amp; a total of 2494 mg/10 mL of n-3 PUFAs</td>
<td>Corn oil contained 4747 mg/10 mL of LA &amp; 92 mg/10 mL of ALA</td>
<td>Oil (no information)</td>
<td>10mL/day</td>
</tr>
<tr>
<td>Dunstan et al., 2008</td>
<td>Yes, although mothers in the Int. group were slightly younger (p=0.047)</td>
<td>Prenatally</td>
<td>From 20 wks.</td>
<td>20 wks. to birth</td>
<td>4.5-5</td>
<td>Fish oil: 1.1g EPA (20:5n-3) and 2.2g DHA per day</td>
<td>Olive oil containing 2.7g n-9 oleic acid</td>
<td>Capsule</td>
<td>Four 1gr/day</td>
</tr>
<tr>
<td>Campoy et al., 2011</td>
<td>Yes</td>
<td>Prenatally &amp; postnatally-in infants</td>
<td>From 22 wks.</td>
<td>22 wks. to 6 months postnatal</td>
<td>10-10.5</td>
<td>Modified Fish Oil (500mg DHA+150mg EPA) &amp; Vit+Min</td>
<td>Fish Oil + 5-MTHF OR 5-MTHF OR Placebo (nature not identified)</td>
<td>Milk-based supplement sachets</td>
<td>15gr one daily dose</td>
</tr>
<tr>
<td>Rytter et al., 2011</td>
<td>Yes</td>
<td>Prenatally only</td>
<td>At 30 wks.</td>
<td>30 wks. to delivery</td>
<td>2-2.5</td>
<td>Fish oil (32% EPA &amp; 23% DHA, together with 2 mg tocopherol/mL added to prevent auto oxidation of EPA &amp; DHA, corresponding to ~2.7g marine n-3 PUFAs/d</td>
<td>Olive oil (72% oleic acid &amp; 12% linoleic acid)</td>
<td>Capsule</td>
<td>Four 1-g/day</td>
</tr>
<tr>
<td>Bergman et al., 2011</td>
<td>Yes</td>
<td>Prenatally &amp;</td>
<td>From 21-37</td>
<td>21-37 wks. &amp;</td>
<td>4-7 (longer)</td>
<td>0.6 fish oil (200)</td>
<td>1) Basic</td>
<td>Flavored &amp;</td>
<td>0.6 g Fish oil</td>
</tr>
<tr>
<td>Primary article</td>
<td>Comparable baseline characteristic</td>
<td>Participants receiving intervention</td>
<td>Timing of Intervention in pregnancy</td>
<td>Intake of Intervention from/until</td>
<td>Duration of intervention (months)**</td>
<td>Fatty acids product***</td>
<td>Placebo/Control</td>
<td>Mode of delivery</td>
<td>Total daily dose</td>
</tr>
<tr>
<td>-----------------</td>
<td>-----------------------------------</td>
<td>-------------------------------------</td>
<td>------------------------------------</td>
<td>---------------------------------</td>
<td>-------------------------------------</td>
<td>------------------------</td>
<td>-----------------</td>
<td>-----------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>al., 2012</td>
<td></td>
<td>postnatally-in mothers</td>
<td>wks.</td>
<td>3 months postnatal (optional)</td>
<td>period for women who continued participation after delivery</td>
<td>mg DHA+60 mg EPA)</td>
<td>Vit/Min 2) Prebiotic FOS³ (to promote a favorable gut flora)</td>
<td>acidified milk-based supplement</td>
<td>(containing 200mg DHA) + 4.5 g FOS + Vit/Min (a skim milk-based, acidified liquid in a 200ml tetra box), daily</td>
</tr>
<tr>
<td>Gonzalez- Casanova et al., 2015</td>
<td>Yes, maternal height was slightly higher in the placebo group (p=0.06)</td>
<td>Prenatally only</td>
<td>From wk. 18-20</td>
<td>From 18-20 wks. to delivery</td>
<td>5-5.5</td>
<td>Algal DHA</td>
<td>Corn-soy oil blend, not containing any DHA or other (n-3) fatty acids</td>
<td>Capsule</td>
<td>400mg (2 capsules taken at the same time)</td>
</tr>
<tr>
<td>Brei et al., 2016</td>
<td>Yes</td>
<td>Prenatally &amp; postnatally-in mothers</td>
<td>From 15 wks.</td>
<td>15 wks. to 4 months postnatal</td>
<td>9.5-10 + 1week</td>
<td>N-3 LCPUFA [1,020 mg (DHA) &amp;180 mg (EPA)]</td>
<td>Standard diet</td>
<td>Capsule</td>
<td>1200mg/day</td>
</tr>
</tbody>
</table>

*Abbreviations: WKS= weeks, MON= months

**Indicates total duration in pregnancy plus after birth either in mothers only or both mother and infant, if applicable

***DHA=Docosahexaenoic acid, EPA=Eicosapentaenoic acid

³Methyltetrahydrofolate

⁴Fructo-oligosaccharide
4.5.4. Risk of bias in studies of maternal fatty acid consumption during pregnancy and prevention of obesity in the offspring

The summary of risk of bias in trials on fatty acid studies is presented in Figure 4.2. The reviewer’s judgment for the risk of bias assessment of fatty acid studies is shown in appendix 4.5.
Random Sequence Generation 43% 57%
Allocation Concealment 43% 43% 14%
Double Blinding 43% 28.5% 28.5%
Blinding of Outcome Assessment 57% 29% 14%
Incomplete Outcome Data 29% 71%
Selective Outcome Reporting 100%
Other Sources of Bias 57% 43%

Figure 4.2. Summary of risk of bias assessment in the included trials of fatty acids and prevention of obesity in the offspring
4.5.4.1. Random sequence generation (selection bias)

Of the seven included trials, three studies were assessed as having a low likelihood of selection bias (Bergmann et al. 2012; Brei et al., 2016; Helland et al., 2008) as the treatment allocation in these studies was carried out using a computer generated randomisation system. The risk of selection bias in the remaining studies was unclear as they had either not reported their method of randomisation or just stated that they had used block randomisation with no further information.

4.5.4.2. Allocation concealment

The allocation concealment was rated as high risk in the Campoy et al. (2011) trial since the randomisation numbers were provided in envelopes left in a closed box for each participating centre. The method of allocation concealment was not clear in three of the studies (Bergmann et al., 2012; Brei et al., 2016; Helland et al., 2008). The remainder kept the allocation concealed from the staff involved with the study (Dunstan et al., 2008; Gonzalez-Casanova et al., 2015; Rytter et al, 2011).

4.5.4.3. Double blinding (performance bias)

The specific intervention in the study conducted by Brei et al. (2016) did not allow a double-blind design and therefore, the study was rated as having a high risk of bias. The study by Rytter et al. (2011) was also deemed as high risk for performance bias since women would know if they were in one of the study’s control groups, as they would not receive any tablets.

A further two studies gave no indication of blinding, of either staff or participants, and were recorded as having an unclear risk (Bergmann et al., 2012; Helland et al., 2008). The final three studies (Campoy et al., 2011; Dunstan et al., 2008; Gonzalez-Casanova et al., 2015) stated that their trial was double-blinded by keeping the codes blinded to research staff throughout the study and also by ensuring an equal appearance and smell for the intervention and placebo supplementations.

4.5.4.4. Blinding of outcome assessment (detection bias)

The study by Brei et al. (2016) was rated as having high risk of bias for blinding of outcome assessment, since the research staff who assessed SFT and infant growth were not blinded to study-group allocation. Two studies were rated as unclear for the blinding of outcome assessment (Campoy et al., 2011; Helland et al., 2008), as there
was no clear information about whether the staff or participants were blinded at the time of assessment, the remainder were rated as low risk of bias (Bergmann et al., 2012; Dunstan et al., 2008; Gonzalez-Casanova et al., 2015; Rytter et al., 2011).

4.5.4.5. Incomplete outcome data (attrition bias)
Completeness of data was ranked as high risk in five studies (Brei et al., 2016; Campoy et al., 2011; Helland et al., 2008; Gonzalez-Casanova et al., 2015; Rytter et al., 2011). One study had a high loss to follow-up and did not specify the reasons for missing participants (Brei et al., 2016). The study by Helland et al. (2008) had a high loss to follow-up with higher non-compliance rate in the intervention group. Similarly, high loss to follow-up was also reported in Campoy et al. (2011), Rytter et al. (2011) and Gonzalez-Casanova et al. (2015) trials with a higher rate being observed in the study by Rytter et al. (2011). The rest of the studies were rated as low risk of bias since a high proportion of their sample participated in the follow-up assessment (Bergmann et al., 2012; Stein et al., 2011).

4.5.4.6. Selective outcome reporting (reporting bias)
All the pre-specified outcomes listed in the published papers were reported and consequently all the included trials were deemed to have a low risk of bias.

4.5.4.7. Other potential sources of bias
Each included study was assessed for other factors that might contribute to additional risk of bias. Three studies were rated as unclear for further risk of bias (Bergmann et al., 2012; Brei et al., 2016; Campoy et al., 2011) since there was no information regarding whether the participants have consumed the fatty acid supplement after the termination of the intervention. The remaining studies were classified as low risk for any further bias.

4.5.5. Meta-analyses of effectiveness of maternal fatty acid consumption during pregnancy and prevention of obesity in the offspring
Pooled results from meta-analyses in the studies that examined the effectiveness of maternal fatty acid supplementations during pregnancy and the prevention of obesity in offspring are presented in the following section. It is worth noting that the ITT analysis was only available data in Bergmann et al. (2012) study and hence, these are included in the meta-analyses. Also, the study conducted by Brei et al., (2016) is
included in sub-group analyses since it had a different comparator to other included studies. Furthermore, length as an outcome of interest for this review was not reported in the trials.

**4.5.5.1. BMI as an outcome for fatty acid intervention**

The effectiveness of fatty acid products during pregnancy on BMI in the offspring was assessed in four studies. Figure 4.3 shows the Forest plot for fatty acids versus placebo in pregnant women for BMI in offspring. The measurement of BMI was described differently in the included studies as below:

**Bergmann et al. (2012):** Length and weight of the children were measured by standardised methods using Harpenden measuring boards, small measuring tapes and calibrated Seca balances; BMI was calculated accordingly. This outcome was reported as point prevalence at 6 years.

**Campoy et al. (2011):** The outcome considered was “BMI, as the secondary endpoint” (undefined). This outcome was reported as point prevalence at 6.5 years.

**Helland et al. (2008):** Growth data as length and weight were measured at follow-up assessment, along with intelligence testing, and the method of measuring the growth outcomes is not defined. This outcome was reported as point prevalence at 7 years.

**Rytter et al. (2011):** Anthropometric measures were taken through a self-administered web-based questionnaire and those who did not answer were invited for a physical examination. The method of measurement was not defined. The outcome was reported as point prevalence at 19 years.

Statistically, these studies were largely homogeneous, with no variation between studies attributable to heterogeneity as opposed to sampling error ($\chi^2=1.53$, $p=0.67$, $I^2=0\%$). The result of meta-analysis did not show an association between maternal intake of fatty acids during pregnancy and BMI in the offspring (Mean Difference (SMD)=-0.013, 95% CI=-0.16-0.18; 520 children) (Figure 4.3).

**Figure 4.3. Forest plot of fatty acids vs. placebo for BMI**

Measure: Continuous: d (Hedges g)
Heterogeneity: $Q = 1.53$; $df = 3$; $p = 0.675$; $I$-squared = 0%; tau-squared = 0.
Random effects model: 0.0136 (-0.162, 0.189)
A sub-group analysis was also conducted including only the studies with similar duration of follow-up (Campoy et al., 2011; Bergmann et al., 2012; Helland et al., 2008). The results did not yield a significant effect (SMD=0.04, 95% CI=-0.18 to 0.25, 337 children) (Forest plot not shown).

**4.5.5.2. BMI-Z as an outcome measure for fatty acid intervention**

Two included studies measured the effect of fatty acids products consumption during pregnancy on BMI-Z in offspring (Figure 4.4). The definition of the outcome in the included studies was as below:

**Bergmann et al. (2012):** The outcome considered was ‘Z-BMI’. The BMIs of the children from birth to 6 years were standardised with age-specific means of the World Health Organisation (WHO) multicentre growth reference study. This outcome was reported as point prevalence at 6 years.

**Gonzalez-Casanova et al. (2015):** The outcome considered was ‘BMI-Z’. BMI was computed by calculating age at measurement from the date of birth, and then converted to age-specific Z-scores using the 2006 WHO reference standards. This outcome was reported as point prevalence at 5 years.

The studies were statistically homogeneous, with no variation between studies ($\chi^2$=0.34, p=0.55, $I^2$=0%). The result of meta-analysis did not show an association between maternal intake of fatty acids during pregnancy and BMI Z-score in the offspring (Mean Difference (SMD)=0.0142, 95% CI=-0.11 to 0.14, 917 children) (Figure 4.4).
4.5.5.3. Weight as an outcome measure for fatty acid intervention

Three included studies measured the effect of fatty acid products consumption during pregnancy on weight in offspring (Figure 4.5). The definition of the outcome in the included studies was as below:

**Bergmann et al. (2012):** Weight (method of measurement undefined), this outcome was reported as point prevalence at 6 years.

**Dunstan et al. (2008):** Weight (method of measurement undefined), this outcome was reported as point prevalence at 2.5 years.

**Gonzalez-Casanova et al. (2015):** Weight (to the nearest 10gr) was measured using a Tanita scale. This outcome was reported as point prevalence at 5 years.

**Helland et al. (2008):** Weight (method of measurement undefined), this outcome was reported as point prevalence at 7 years.

The studies were statistically homogeneous ($\chi^2=0.97, p=0.80, I^2=0\%$). The result of meta-analysis did not show an association between maternal intake of fatty acids during pregnancy and weight in the offspring (SMD=-0.01, 95% CI=-0.13-0.10; 934 children).
Figure 4.5. Forest plot of fatty acids vs. placebo for weight

Measure: Continuous: d (Hedges g)
Heterogeneity: Q = 0.974; df = 3; p = 0.808; I-squared = 0%; tau-squared = 0.
Random effects model: -0.0142 (-0.131, 0.103)

<table>
<thead>
<tr>
<th>Outcome: Weight</th>
<th>Fatty acids Mean (SD)</th>
<th>Placebo Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bergmann (n=41/74)</td>
<td>22.4 (3.1)</td>
<td>22.3 (2.9)</td>
</tr>
<tr>
<td>Dunstan (n=33/39)</td>
<td>14.5 (2.0)</td>
<td>14.1 (2.0)</td>
</tr>
<tr>
<td>Gonzalez-Casanova (n=369/370)</td>
<td>10.4 (1.1)</td>
<td>10.4 (1.2)</td>
</tr>
<tr>
<td>Helland (n=82/61)</td>
<td>26.8 (4.1)</td>
<td>27.0 (4.1)</td>
</tr>
<tr>
<td><strong>Subtotal</strong> (n=525/409)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In addition, an additional analysis was conducted including the study conducted by Brei et al. (2016), which defined “standard diet” as its comparator. The outcome in this study was measured as below:

**Brei et al. (2016):** The weight was measured to the nearest 100g using a standard flat scale (Seca Clara 803), with the child in a standing position. The outcome was reported as point prevalence at 5 years.

As Figure 4.6 shows, the studies were largely homogenous ($\chi^2=2.85$, p=0.58, $I^2=0\%$). The meta-analysis did not alter the results greatly, indicating that there was no association between prenatal fatty acid supplementation during pregnancy and weight in the offspring (SMD=0.010, 95% CI=-0.101-0.12, 1,183 children).

Figure 4.6. Forest plot of fatty acids vs. control for weight

Measure: Continuous: d (Hedges g)
Heterogeneity: Q = 2.85; df = 4; p = 0.583; I-squared = 0%; tau-squared = 0.
Random effects model: 0.0106 (-0.101, 0.122)
4.5.5.4. Height as an outcome measure for fatty acid intervention

The meta-analysis on the maternal consumption of fatty acids during pregnancy and its effectiveness on height in the offspring is shown in Figure 4.7. In total, four studies were included in the meta-analysis. The outcome considered within the included studies were as below:

**Bergmann et al. (2012):** Height (method of measurement undefined), this outcome was reported as point prevalence at 6 years.

**Dunstan et al. (2008):** Height (method of measurement undefined), this outcome was reported as point prevalence at 2.5 years.

**Gonzalez-Casanova et al. (2015):** Height (to the nearest 1mm) was measured using a Seca stadiometer. This outcome was reported as point prevalence at 5 years.

**Helland et al. (2008):** Height (method of measurement undefined), the outcome was reported as point prevalence at 7 years.

Statistically, these studies were largely homogeneous, with no variation between studies attributable to heterogeneity ($\chi^2=1.51$, $p=0.68$, $I^2=0\%$). The result of meta-analysis did not show an association between maternal intake of fatty acids during pregnancy and height in the offspring (SMD=$-0.042$, 95% CI=$-0.159$-$0.07$; 1,069 children) (Figure 4.7).
Figure 4.7. Forest plot of fatty acids vs. placebo for height

Measure: Continuous: d (Hedges g)
Heterogeneity: Q = 1.51; df = 3; p = 0.68; I-squared = 0%; tau-squared = 0.
Random effects model: -0.0421 (-0.159, 0.0751)

<table>
<thead>
<tr>
<th>Outcome: Height</th>
<th>Fatty acids Mean (SD)</th>
<th>Placebo Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bergmann (n=41/74)</td>
<td>119.2 (5.3)</td>
<td>119.6 (4.6)</td>
</tr>
<tr>
<td>Dunstan (33/39)</td>
<td>93.8 (3.8)</td>
<td>93.3 (4.6)</td>
</tr>
<tr>
<td>Gonzalez-Casanova (n=369/370)</td>
<td>108.3 (4.4)</td>
<td>108.4 (4.5)</td>
</tr>
<tr>
<td>Helland (n=82/61)</td>
<td>127.5 (5.5)</td>
<td>128.6 (5.0)</td>
</tr>
<tr>
<td>Subtotal (n=525/544)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A sub-group analysis was also conducted including the study conducted by Brei et al. (2016) that defined “standard diet” as its comparator. The outcome in this study was defined as below:

**Brei et al. (2016):** Height was measured to the nearest 0.5cm using a stadiometer, with the child in a standing position. The outcome was reported as point prevalence at 5 years.

As Figure 4.8 shows, there was moderate level of heterogeneity between the studies (χ²=5.17, p=0.27, I²=22.6%). The meta-analysis did not alter the results greatly, indicating the result of meta-analysis did not show any association between maternal intake of fatty acids during pregnancy and height in the offspring (SMD=0.001, 95% CI=-0.147-0.15, 1,183 children).

Figure 4.8. Forest plot of fatty acids vs. control for height

Measure: Continuous: d (Hedges g)
Heterogeneity: Q = 5.17; df = 4; p = 0.27; I-squared = 22.6%; tau-squared = 0.00701.
Random effects model: 0.00157 (-0.147, 0.151)
<table>
<thead>
<tr>
<th>Outcome: Height</th>
<th>Fatty acids Mean (SD)</th>
<th>Placebo Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bergmann (n=41/74)</td>
<td>119.2 (5.3)</td>
<td>119.6 (4.6)</td>
</tr>
<tr>
<td>Brei (n=58/56)</td>
<td>112.2 (4.8)</td>
<td>110.7 (4.0)</td>
</tr>
<tr>
<td>Dunstan (33/39)</td>
<td>93.8 (3.8)</td>
<td>93.3 (4.6)</td>
</tr>
<tr>
<td>Gonzalez-Casanova (n=369/370)</td>
<td>108.3 (4.4)</td>
<td>108.4 (4.5)</td>
</tr>
<tr>
<td>Helland (n=82/61)</td>
<td>127.5 (5.5)</td>
<td>128.6 (5.0)</td>
</tr>
</tbody>
</table>

4.5.5.5. Sum of SFT as an outcome measure for fatty acid intervention

The outcome measure as a sum of SFT in children was measured in two of the included studies (Bergmann et al., 2012 and Brei et al., 2016), which have introduced different comparators as “placebo” and “standard diet” respectively. A meta-analysis was performed including these two studies investigating the effect of prenatal intake of fatty acids on sum of SFT in the offspring (Figure 4.9). Measurement of the outcome in the included trials was described as below:

**Bergmann et al. (2012):** The outcome considered was ‘sum of SFT (mm)’. SFT was measured with a Holtain caliper at the mid tricipital, the subscapular, and the suprailliac measuring point. This outcome was reported as point prevalence at 6 years.

**Brei et al. (2016):** SFTs, as a primary outcome, was measured in triplicate with the use of a Holtain caliper at four different body sites on the left body axis: triceps, biceps, subscapular and suprailliac. Measurements were performed at 2, 3, 4 and 5 years of age at the study centre or at the family’s home. For each site, the mean of the three measurements was used for the SFT value and the sum of the 4 SFTs was calculated. This outcome was reported as point prevalence at 5 years.

The studies were largely heterogeneous ($\chi^2=2.67, P=0.10, I^2=62.6\%$). The result of meta-analysis did not show an association between maternal intake of fatty acids during pregnancy and SFT in the offspring (SMD=0.09, 95% CI=-0.33-0.53, 227 children) (Figure 4.9).
Figure 4.9. Forest plot of fatty acids vs. control for sum of SFT

Measure: Continuous: d (Hedges g)
Heterogeneity: Q = 2.67; df = 1; p = 0.102; I-squared = 62.6%; tau-squared = 0.062.
Random effects model: 0.0968 (-0.339, 0.533)

<table>
<thead>
<tr>
<th>Outcome: Sum of SFT</th>
<th>Fatty acids Mean (SD)</th>
<th>Placebo Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bergmann (n=41/74)</td>
<td>23.2 (7.1)</td>
<td>21.1 (6.1)</td>
</tr>
<tr>
<td>Brei (n=57/55)</td>
<td>23.9 (4.7)</td>
<td>24.5 (5.0)</td>
</tr>
<tr>
<td>Subtotal (n=98/129)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.5.6. Discussion of the evidence synthesis of maternal fatty acid consumption during pregnancy and prevention of obesity in the offspring

4.5.6.1. Summary of main results

This systematic review summarised data from seven RCTs of prenatal intake of fatty acids for prevention of childhood adiposity that included a total of 1,647 children with follow-up ranging from 1.5 to 19 years (mean 7.28 years). Method of allocation concealment was rated unclear in three studies, with one study judged as high risk. Also, over half of the studies (71%) were deemed as high risk for loss to follow-up.

The findings from this systematic review and meta-analysis do not provide evidence of a protective association between intake of fatty acids during pregnancy and subsequent development of a number of obesity measures in children including BMI, BMIZ, weight, height, length, sum of SFT. These results, however, need to be considered with caution due to the risk of bias in the trials, limited number of studies, a small sample size in most studies, and the statistical heterogeneity observed for some outcomes i.e. height and SFT.

It is also worth mentioning that with the exception of the study by Brei et al., (2016), the other five included trials were primarily designed to assess outcomes other than obesity in childhood, and reported growth measures as their secondary outcomes in further follow-up assessments. In an open label trial, Brei and colleagues (2016) applied a combined intervention approach of fish oil capsules and AA-balanced diet...
(ratio of n-6/n-3) during pregnancy, and introduced a number of measures for adiposity outcomes such as mean of SFT and fat distribution in infants by 5 year of age. Data from this trial did not demonstrate a significant difference between the study arms for adiposity outcomes in children. It is important to note that the study had a high non-participation rate at the 5 years follow-up.

4.5.6.2. Overall completeness and applicability of evidence
No evidence was found that fatty acid supplementation during pregnancy is effective for the prevention of adiposity in the offspring. The heterogeneity between the trials limited the findings and random effect models were used to pool the results. Heterogeneity between studies originated from varied dosage and timing of intervention, variability of comparators, different methods for reporting the outcome measures across studies, diverse locations/settings and the follow-up duration. One study (Gonzalez-Casanova et al., 2015) also used DHA, isolated from algal, which may act differently from fish oil. It was not possible to conduct stratified analyses for the type, dosage and timing of intervention to explore the differences caused by the relatively small number of trials in each group. In addition, although most studies recruited a relatively large sample, only a small number of participants were approached at their follow-up.

4.5.6.3. Quality of evidence
Overall, the trials were at moderate to high risk of bias, for the summary of risk of bias assessment (Figure 4.2). A large proportion of studies were rated as unclear in many risk of bias domains, and some had high risk of bias for individual quality domains (Figure 4.2). High loss to follow-up was the main concern in five of the included trials (71%) and the number of studies and participants contributing to the meta-analyses is also an issue that might downgrade the quality of the body of evidence.

4.5.6.4. Strength and weakness of this systematic review for fatty acid consumption during pregnancy
The main distinction of this systematic review is that it includes only trials that started intake of fatty acids during pregnancy, thus, crucially, enabling us to isolate the effect of prenatal intake of fatty acids for prevention of childhood obesity. An additional strength is that the systematic review followed an a priori published protocol, using a
comprehensive search strategy that allowed complete coverage of all the relevant literature including citation databases, trial registries and conference proceedings. Moreover, a range of obesity measures were the focus of the present review and the most up-to-date results from the trials, reported as the longest available follow-up data, are included in the meta-analyses.

A limitation of the review is that we made some changes to the protocol. Sub-group analyses were not conducted as planned for the type, duration and dosage of intervention, owing to the small number of studies that could contribute in meta-analysis. Nevertheless, the established methodological guidelines from the protocol were largely followed, and there was a clear rational for deviations from the protocol. As a consequence of these changes, data were pooled from trials conducted in diverse populations, initially designed to account for the different outcome and quality of studies. It is worth noting that sub-group analyses conducted in the current systematic review might be susceptible to type II errors due to relatively small sample sizes in trials; however no statistical heterogeneity was introduced in most of the meta-analyses conducted. Furthermore, the limited number of studies did not allow formal assessment for publication bias.

4.5.6.5. Agreement and disagreement with other reviews

As described in section 4.3.2.2, there have been few systematic reviews for the use of fatty acids during pregnancy for prevention of childhood obesity. Most of these provided a narrative description of the included studies, with the exception of one meta-analysis that reported maternal and neonatal outcomes at birth only (Imhoff-Kunsch et al., 2012). One systematic review and meta-analyses by Stratakis and colleagues (2014) focused on longer-term adiposity outcomes in children and used different inclusion criteria from the current review in terms of the timing of intervention where they included studies that administered fatty acids supplements prenatally and/or exclusively in lactation. They also defined a few obesity outcomes and conducted the meta-analyses only for BMI with subgroup analyses for age groups (<5, 6-12 and >13 years) and the timing of intervention i.e. pregnancy, pregnancy and lactation, lactation. In addition, Stratakis and colleagues included BMI in units of either kg/m² or z-score in the meta-analyses. Our systematic review provides an update with that of Startakis et al. (2014), and although our results are not directly
comparable with their review, the current findings are in agreement with the previous review that concludes fatty acid supplementation during pregnancy does not protect against adiposity-related measures in children. Similarly, they have also reported methodological shortcomings including number of studies, small sample sizes and attrition bias.

4.6.5.6. Author’s conclusion

Implications for practice
The results of the current systematic review do not provide an evidence for the prevention of obesity in the offspring by prenatal fatty acid intake when compared with placebo/no treatment. Due to the high heterogeneity between studies along with small sample size and large attrition at follow-ups, the effects of fatty acid supplementation during pregnancy for prevention of childhood adiposity in long-term remains unclear.

Implications for research
Taking the volume and quality of the available evidence into account, the effect of prenatal intake of fatty acids for prevention of childhood obesity needs to be further investigated in large, high quality RCTs. Given that only one included study had been established to explicitly examine the effect of prenatal consumption of fatty acids on obesity in offspring, such RCTs need to be dedicated trials designed specifically to examine this question. Trials should also consider the effect of n-6/n-3 ratio in the dietary intervention and rather than increasing n-3 LC-PUFA in isolation to determine the role of the balance of fatty acid intake in maternal diet. Using combined methods of anthropometric and SFT measurements as well as more precise measures such as MRI and ultrasound will also allow for more accurate estimation of adipose tissue deposition in children.

The optimal timing of fatty acid intervention is another key factor that needs to be further investigated. The first appearance of adipocytes in the human foetus occurs in second trimester of pregnancy, between 14-16 weeks of gestation (Ailhaud & Hauner, 2004). Further research is required to determine the critical window for programming of offspring adipose tissue. Baseline level of DHA in pregnant women, type and optimal dose of LC-PUFA, as well as the choice of control regimens, are elements
that need to be considered in further trials. More importantly, additional rigorous strategies are needed to minimise the low participation rate at follow-up assessments. Recruiting fewer participants or high attrition rates in interventions with ω-3 PUFAs could lead to significantly varied findings and thus bias in impact of fatty acids, as highlighted in a recent study (Yelland, Makrides, McPhee, Quinlivan, & Gibson, 2016). Therefore, significant research by conducting longitudinal studies with adequate sample size and repeated measurements is required to provide a strong evidence with which to determine the effect of fatty acids intake during pregnancy on obesity in offspring.

One might also argue that the existing evidence is applicable only to the populations in which these studies were undertaken. Given that the majority of the studies were conducted in developed countries, it remains a priority to also understand the effectiveness of these interventions for childhood obesity among the underreported populations.
4.5.7. Description of included studies of maternal probiotic consumption during pregnancy and prevention of obesity in the offspring

In total four studies (with a total of 1,610 children) examined the impact of probiotic supplementation during pregnancy on the development of obesity in offspring. The characteristics of the included trials, their companion papers and study population are presented in Table 4.5. Studies were conducted in Finland, Denmark, Sweden and New Zealand.

Only one study was specifically designed to investigate the impact of perinatal probiotic intervention on the development of overweight and obesity in children and reported the 10 year follow-up of the original study (Luoto, Kalliomäki, Laitinen, & Isolauri, 2010). In contrast, the other included studies were originally planned to investigate the effect of maternal supplementation with probiotics during pregnancy on developing allergic diseases in offspring and additionally reported the anthropometric/growth measures of children in their extended follow-up along with the allergic outcomes.

One study used a combination of a probiotic and a prebiotic in pregnant women (Kuitunen, et al., 2009) and the remainder applied probiotics only (Abrahamsson et al., 2013; Luoto et al., 2010; Wickens et al., 2013). All studies selected their study sample from families with a reported history of atopic diseases.

The longest follow-up period was 10 years, in the study conducted by Luoto et al. (2010), followed by 7 and 6 years in the studies by Abrahamsson et al. (2013) and Wickens et al. (2013) respectively. The largest study sample was reported in Kuitunen et al. (2009) with 1,223 mothers enrolled followed by Wickens et al. (2013) with 511 pregnant mothers at enrolment. The smallest sample size was observed in study conducted by Luoto et al. (2010) with 159 mothers randomised at recruitment.

Compliance with the treatment was assessed by interviews/stool examination and counting of unused supplements. Method of adherence to intervention was not reported in the study conducted by Luoto et al. (2010).
### Table 4.5. Characteristics of the included trials and study population of probiotics and prevention of obesity

<table>
<thead>
<tr>
<th>Primary article</th>
<th>Companion articles*</th>
<th>Country, enrolment period</th>
<th>Trial type*</th>
<th>Study intervention &amp; comparator</th>
<th>No. of participants**</th>
<th>No. at last F-U***</th>
<th>Time points measured</th>
<th>Age at last F-U</th>
<th>Sample: high risk of Atopy</th>
<th>Outcomes reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Kuitunen, et al., 2009)</td>
<td>(Kukkonen et al., 2007, 2008; Kukkonen, Nieminen, Poussa, Savilahti, &amp; Kuitunen, 2006a)</td>
<td>Finland 2000-03</td>
<td>PC-RCT</td>
<td>Mixed Probiotic &amp; placebo</td>
<td>1,223 mothers</td>
<td>891 (445 vs. 446)</td>
<td>3, 6, 12, 24 months</td>
<td>5yrs.</td>
<td>Yes</td>
<td>-Weight (kg) -Height (cm)</td>
</tr>
<tr>
<td>(Luoto, et al., 2010)</td>
<td>(Luoto, Laitinen, Nermes, &amp; Isolauri, 2010)</td>
<td>Denmark no information</td>
<td>PC-RCT</td>
<td>Two Probiotics supplement &amp; control</td>
<td>159 mothers</td>
<td>113: 59 vs. 54</td>
<td>3, 6, 12 &amp; 24 months; 4, 7 &amp; 10yrs</td>
<td>10yrs.</td>
<td>Yes</td>
<td>-BMI (kg/m²) -Obesity -Overweight</td>
</tr>
<tr>
<td>(Abrahamsson, et al., 2013)</td>
<td>(Abrahamsson et al., 2007; Böttcher, et al., 2008; Forsberg, et al., 2014)</td>
<td>Sweden 2001-03</td>
<td>PC-RCT</td>
<td>Lactobacillus reuteri &amp; placebo</td>
<td>232 mothers</td>
<td>184 (94 vs. 90)</td>
<td>2 &amp; 7 yrs.</td>
<td>7yrs.</td>
<td>Yes</td>
<td>-Weight (kg) -Height (cm)</td>
</tr>
<tr>
<td>(Wickens et al., 2013)</td>
<td>(Dekker et al., 2009; Prescott et al., 2008; Wickens et al., 2008, Wickens et al., 2012)</td>
<td>New Zealand 2004-05</td>
<td>PC-RCT</td>
<td>2 diff mixed probiotic arms &amp; placebo</td>
<td>511 mothers</td>
<td>422(134 vs. 144 vs. 144)</td>
<td>2, 4 &amp; 6yrs.</td>
<td>6yrs.</td>
<td>Yes</td>
<td>-BMI (kg/m²) -Obesity -Overweight</td>
</tr>
</tbody>
</table>

---

*Published data and conference presentations. No unique data were extracted from conference abstracts

*Placebo Controlled-Randomised Controlled Trial

*Indicates the number at randomisation, where recruitment has occurred prenatally

**Follow-up
Table 4.6. shows the details of the probiotic interventions and placebo used in included trials. In all studies the intervention and placebo groups had comparable baseline characteristics at recruitment and also at birth. It is worth noting that the Abrahamsson et al. (2013) study reported higher antibiotic prescription during the first year of life in the intervention vs. placebo group (p=0.03).

In all studies, the probiotic interventions were continued after pregnancy either in mothers only or with both mothers and their infants for a period of time (Table 4.6). The longest duration of intervention was 25 months (Wickens et al., 2013) and the shortest was 6.5-7 months (Kuitunen et al., 2009 & Luoto et al., 2010). In one study, a single strain of probiotics was used as the intervention (Abrahamsson et al., 2013) whereas a mixed strain of probiotics was employed in the other included studies. For the purpose of this systematic review, all the probiotic intervention studies, whether they have used mixed or single strains of probiotics, have been grouped together under one umbrella as “any probiotics”.

One study included two active intervention trial arms by employing different mixed strains of probiotics in each intervention group and comparing these with a placebo (Wickens et al., 2013). For reporting purposes and as recommended by the Cochrane Handbook (Higgins et al., 2011b), data from the two different active intervention arms were pooled when entered into meta-analysis. On a request for the anthropometric measures from the author, the combined data for intervention arms were also sought.

All studies used placebo as their comparator/control. Probiotic preparations included capsules and oil drops. In the study by Luoto et al. (2010), participants of both probiotic and placebo groups also received an intensive dietary counseling intervention at every study visit by a nutritionist. Women were advised to follow a diet complying with current recommendations, combined with conventional food products with favourable fat and fibre contents for use at home.
## Table 4.6. Characteristics of probiotic interventions in the included trials for prevention of obesity

<table>
<thead>
<tr>
<th>Primary article</th>
<th>Comparable baseline characteristic</th>
<th>Participants receiving intervention</th>
<th>Timing of Intervention in pregnancy</th>
<th>Intake of intervention from/until†</th>
<th>Duration of intervention (months)**</th>
<th>Probiotic organism***</th>
<th>Placebo</th>
<th>Mode of delivery</th>
<th>Total daily dose (colony forming units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kuitunen et al., 2009</td>
<td>Yes</td>
<td>Prenatally and postnatally in infants</td>
<td>From 36wks. of gestation</td>
<td>6 months postnatal</td>
<td>6.5-7</td>
<td>LC705 + LC705 +bb99 + Pf</td>
<td>Micro-crystalline cellulose</td>
<td>Capsule</td>
<td>5 × 10⁹, 5 × 10⁸, 2 × 10⁹ CFU/day, twice a day</td>
</tr>
<tr>
<td>Luoto et al., 2010</td>
<td>Yes</td>
<td>Prenatally &amp; postnatally in mothers &amp; infants</td>
<td>4wks before expected delivery</td>
<td>34-36wks. to 6 months postnatal</td>
<td>6.5-7</td>
<td>LGG &amp; BBL + intensive dietary counseling</td>
<td>Microcrystalline cellulose &amp; dextrose anhydrate</td>
<td>Capsule</td>
<td>10⁷ per day each strain</td>
</tr>
<tr>
<td>Abrahamsson et al., 2013</td>
<td>Yes, higher Antibiotic prescription during the first year of life in Int. vs. placebo in 1st year of life</td>
<td>Prenatally and postnatally in infants</td>
<td>From 36+0 wks.</td>
<td>36 wks. to 12 months postnatal</td>
<td>12.5-13</td>
<td>L. reuteri</td>
<td>Same oil without any bacteria</td>
<td>Oil drops</td>
<td>1 × 10⁸ CFU/day, 5 drops daily</td>
</tr>
<tr>
<td>Wickens et al., 2013</td>
<td>Yes</td>
<td>Prenatally &amp; postnatally in mothers &amp; infants</td>
<td>From 35wks. gestation</td>
<td>35wks to 2yrs. postnatal</td>
<td>25-25.5</td>
<td>BA HN019 or L. rhamnosus HN001</td>
<td>Dextran, salt, and a yeast extract</td>
<td>Capsule</td>
<td>9 × 10⁹, 6 × 10⁸ CFU/day, daily</td>
</tr>
</tbody>
</table>

*Indicates total duration in mother, infant or both, whichever is applicable
†Indicates total duration in pregnancy plus after birth either in mother only or both mother and infants, if applicable
***DHA=Docosahexaenoic Acid, PUFA=Poly-Unsaturated Fatty Acid, BCSO=Blackcurrant Seed Oil, LA=Linoleic Acid
4.5.8. Risk of bias in studies of maternal probiotic consumption during pregnancy and prevention of obesity in the offspring

Figure 4.10 shows the summary of risk of bias assessment in trials of probiotic studies. Appendix 4.6 shows the reviewer’s judgment of the risk of bias assessment for probiotic studies.
<table>
<thead>
<tr>
<th>Short Title</th>
<th>Random Sequence Generation</th>
<th>Allocation Concealment</th>
<th>Double Blinding</th>
<th>Blinding of Outcome Assessment</th>
<th>Incomplete Outcome Data</th>
<th>Selective Outcome Reporting</th>
<th>Other Sources of Bias</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abrahamsson (2013)</td>
<td>?</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>?</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Wickens (2013)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>?</td>
<td>+</td>
</tr>
</tbody>
</table>

**Figure 4.10. Summary of risk of bias assessment in the included trials of probiotics and prevention of obesity in the offspring**

Low risk of bias  
High risk of bias  
Unclear risk of bias  

4.5.8.1. Random sequence generation (selection bias)

Two studies were rated as low risk of bias for their randomisation method (Kuitunen et al., 2009; Wickens et al., 2013). The remaining two studies were assessed as having an unclear risk of bias (Abrahamsson et al., 2013; Luoto et al., 2010) where it has been generally stated as randomly assigned with no details on the method of randomisation.

4.5.8.2. Allocation concealment

The study by Luoto et al. (2010) was rated as ‘unclear’ as there was no information on how participants were allocated. Methods for concealment of allocation were reported in the remaining studies (Abrahamsson et al., 2013; Kuitunen et al, 2009; Wickens et al., 2013) and were subsequently classified as having a low risk of bias for allocation concealment.

4.5.8.3. Double Blinding (performance bias)

There was no blinding of either staff or participants at the time of extended follow-up in two studies (Abrahamsson et al., 2013; Wickens et al., 2013). The rest of the studies were classified as low risk for their performance bias.

4.5.8.4. Blinding of outcome assessment (detection bias)

One study was rated as having high risk of bias for blinding of outcome assessment (Abrahamsson et al., 2013), as the extended follow-up study was only single-blinded to the investigator(s). One study was rated as unclear for the blinding of outcome assessment (Kuitunen et al., 2009) and the remainder were classified as low risk (Luoto et al., 2010; Wickens et al., 2013).

4.5.8.5. Incomplete outcome data (attrition bias)

Completeness of data was ranked as high risk in two studies. One study had a high loss to follow-up and did not specify the reasons for missing participants (Abrahamsson et al., 2013) and the study by Wickens et al. (2013) used imputed analysis for a number of the reported outcomes. Two studies (Kuitunen et al., 2009; Luoto et al., 2010) were rated as unclear as they did not specify the reasons for loss to follow-up.
4.5.8.6. Selective outcome reporting (reporting bias)

Three studies were rated as unclear since the original study was primarily designed to assess allergic outcomes in children (Abrahamsson et al., 2013; Kuitunen et al., 2009; Wickense et al., 2013) and there is no indication in the manuscript as to whether the anthropometric measures are defined as the secondary outcomes. The study by Luoto et al. (2010) specified the obesity measures as primary outcomes in their protocol/manuscript and was classified as low risk of bias.

4.5.8.7. Other potential sources of bias

There were no concerns regarding any other sources of bias in two of the included trials which were therefore assessed as low risk (Kuitnen et al., 2009; Wickens et al., 2013). One study was rated as being at high risk of further bias (Abrahamsson et al., 2013) since the participants reported that they continued to consume the study intervention product after delivery despite this not being part of the study protocol. The study by Luoto et al. (2010) was rated as having an unclear risk of bias since there is no information whether children in the intervention group have consumed the probiotics after the study termination.

4.5.9. Meta-analyses of effectiveness of maternal probiotic consumption during pregnancy and prevention of obesity in the offspring

Pooled results from meta-analysis in the studies that examined the effectiveness of probiotic supplementations for the prevention of obesity in offspring are presented in the following section. Since the studies were inconsistent for the reported outcomes on anthropometric measures, all four authors were contacted requesting ‘Mean (Standard Deviation (SD))’ of growth outcomes i.e. crude/unadjusted BMI, weight, height. Two authors (Abrahamsson 2013; Kuitunnen 2009) did not provide the requested data. One author provided only the unadjusted BMI at different follow-up time points (Luoto et al., 2010). Wickens et al. (2013) contributed data on BMI, weight and height for individual intervention arms and also, as requested, for combined intervention arms. For this systematic review, the data for combined probiotic intervention arms were included in meta-analyses. It is worth noting that in the study by Wickens et al. (2013), there were no significant statistical differences in the reported anthropometric measures for individual probiotic arms. Furthermore, the study by Luoto et al. (2010) reported the anthropometric measures at 2, 4, 7 and 10
years and the follow-up data at 7 years was included in meta-analyses as it is more comparable to the reported follow-up times in the other included studies. Data on anthropometric measures at 10 years follow-up for this study are reported separately. The definition of the outcomes included in individual meta-analysis is presented in the following sections.

4.5.9.1. BMI as an outcome measure for probiotic intervention

The effectiveness of probiotic supplementations during pregnancy on BMI in children was assessed in three studies. Figure 4.11 shows the forest plot for probiotics versus placebo in pregnant women on BMI in offspring. Measurement of BMI in the included studies was described as below:

Luoto et al. (2010): The outcome considered was “BMI, crude”. The BMI (in kg/m$^2$) was calculated as weight in kilograms divided by height in meters squared and approximated to one decimal place. This outcome was reported as point prevalence at 7 years.

Wickens et al. (2013): The outcome considered was “BMI, combined probiotic arms”. BMI was defined as weight (kg)/height (m$^2$). This outcome is reported as point prevalence at 6 years.

Statistically, the studies were largely homogenous ($\chi^2=0.002$, $P=0.95$, $I^2=0\%$). The result of meta-analysis did not show an association between maternal intake of probiotics during pregnancy and BMI in the offspring (SMD=0.01, 95%CI=-0.17-0.19, 480 children).

Figure 4.11. Forest plot of probiotics vs. placebo for BMI

Measure: Continuous: d (Hedges g)
Heterogeneity: $Q = 0.00278$; df = 1; $p = 0.958$; I-squared = 0%; tau-squared = 0.
Random effects model: 0.0104 (-0.176, 0.197)
<table>
<thead>
<tr>
<th>Outcome: BMI</th>
<th>Probiotics Mean (SD)</th>
<th>Placebo Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luoto (n=54/59)</td>
<td>16.88 (2.04)</td>
<td>16.84 (2.09)</td>
</tr>
<tr>
<td>Wickens (n=245/127)</td>
<td>15.88 (1.38)</td>
<td>15.87 (1.20)</td>
</tr>
<tr>
<td>Subtotal: (n=299/181)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.5.9.2. Weight as an outcome measure for probiotic intervention

Three included studies measured the effect of probiotic consumption during pregnancy on weight in offspring (Figure 4.12). Weight was measured as described below:

Abrahamsson et al. (2013): The outcome considered was ‘Weight (kg), mean (SD)’, undefined. This outcome is reported as point prevalence at 7 years. The authors presented “weight, mean (95% CI)” and the SD was calculated from 95% CI using the formula below, as detailed in the Cochrane handbook (Higgins & Deeks 2011):

\[
\text{SD} = \sqrt{N \times (\text{upper limit} - \text{lower limit}) / 3.92}
\]

Kuitunen et al. (2009): The outcome considered was ‘Weight (kg), mean (SD)’, undefined. The percentage of national reference in the intervention and placebo groups was also reported as 2.64 (9.18) and 1.58 (8.71) respectively. This outcome was reported as point incidence at 5 years.

Wickens et al. (2013): The outcome considered was ‘Weight (kg), mean (SD)’ for combined probiotic groups, undefined. This outcome was reported as point prevalence at 6 years.

There was no statistical heterogeneity between the included trials (\(\chi^2=0.35, P=0.83, I^2=0 \%\)). The result of meta-analysis did not show an association between maternal intake of probiotics during pregnancy and weight in the offspring (SMD=0.06, 95% CI=-0.03-0.17, 1,421 children).

Figure 4.12. Forest plot of probiotics vs. placebo for weight

Measure: Continuous: d (Hedges g)
Heterogeneity: Q = 0.353; df = 2; p = 0.838; I-squared = 0%; tau-squared = 0.
Random effects model: 0.0659 (-0.0396, 0.171)
<table>
<thead>
<tr>
<th>Outcome: Weight</th>
<th>Probiotic Mean (SD)</th>
<th>Placebo Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abrahamsson (n=81/77)</td>
<td>25.4 (3.9)</td>
<td>25.5 (4.7)</td>
</tr>
<tr>
<td>Kuitunen (n=445/446)</td>
<td>19.7 (2.6)</td>
<td>19.5 (2.5)</td>
</tr>
<tr>
<td>Wickens (n=245/127)</td>
<td>22.61 (3.00)</td>
<td>22.39 (2.83)</td>
</tr>
<tr>
<td>Subtotal (n=771/650)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.5.9.3. Height as an outcome measure for probiotic intervention

The meta-analysis on the maternal consumption of probiotics during pregnancy and their effectiveness on height in the offspring is shown in Figure 4.13. In total, three studies were included in the meta-analysis. Measurement of height in the included trials was as below:

**Abrahamsson et al. (2013):** The outcome considered was ‘Height (cm), mean (SD)’, (undefined). This outcome was reported as point prevalence at 7 years. The authors presented “height, mean (95% CI)” and SD was obtained from 95% CI as stated in section 4.5.9.2. This outcome was reported as point prevalence at 7 years.

**Kuitunen et al. (2009):** The outcome considered was ‘Height (cm), mean (SD)’, (undefined). Also, the SD of national reference in the intervention and placebo groups was reported as 0.38 (0.99) and 0.38 (0.99) respectively. This outcome was reported as point incidence at 5 years.

**Wickens et al. (2013):** The outcome considered was ‘Height (cm), mean (SD)’ for combined probiotic groups (undefined). This outcome was reported as point prevalence at 6 years.

Statistically, studies were largely homogenous ($\chi^2=0.76$, $P=0.68$, $I^2=0\%$). The result of meta-analysis did not show an association between maternal intake of probiotics during pregnancy and height in the offspring (SMD=0.03, 95% CI=-0.07-0.14, 1,421 children) (Figure 4.13).

**Figure 4.13. Forest plot of probiotics vs. placebo for height**

Measure: Continuous: d (Hedges g)
Heterogeneity: $Q = 0.768$; df = 2; $p = 0.681$; $I$-squared = 0%; tau-squared = 0.
Random effects model: 0.035 (-0.0704, 0.14)
4.5.9.4. Obesity as an outcome measure for probiotic intervention

In total two studies were included in a meta-analysis measuring the effect of probiotic intake during pregnancy on obesity in the offspring (Figure 4.14). Measurement of obesity in the included trials was as below:

Luoto et al. (2010): The outcome considered was ‘Obesity, number (%)’. The weight status, in calculating BMI, was established by the International Obesity Task Force criteria for overweight and obesity, which identifies BMI values for each age associated with a predicted BMI of 25 or 30 at the age of 18 years. Obesity at 7 years was defined according to the international cut-off values $20.51 \text{ kg/m}^2$ for girls and $20.63 \text{ kg/m}^2$ for boys. This outcome was reported as point prevalence at 7 years.

Wickens et al. (2013): The outcome considered was ‘Obesity, number (%)’ and was defined with gender and age-specific cut-off values. This outcome was reported as point prevalence at 6 years.

A moderate level of statistical heterogeneity was observed between the included trials ($\chi^2=2.26$, $P=0.13$, $I^2=55.7\%$). The result of meta-analysis did not show an association between maternal intake of probiotics during pregnancy and developing obesity in the offspring ($RR=0.38$, 95% CI=0.03-3.91, 485 children).

Figure 4.14. Forest plot of probiotics vs. placebo for obesity

Measure: Binary: risk ratio
Heterogeneity: $Q = 2.26$; $df = 1$; $p = 0.133$; I-squared = 55.7%; tau-squared = 1.72.
Random effects model: 0.38 (0.0369, 3.91)
4.5.9.5. Overweight as an outcome measure for probiotic intervention

In total two studies were included in a meta-analysis measuring the effect of probiotic consumption during pregnancy on overweight in offspring (Figure 4.15). Measurement of overweight in the included trials was defined as below:

**Luoto et al. (2010):** The outcome considered was ‘Overweight, number (%)’. The weight status, in calculating BMI, was established by the International Obesity Task Force criteria for overweight and obesity, which identifies BMI values for each age associated with a predicted BMI of 25 or 30 at the age of 18 years. At 7 years, a child was considered overweight if the BMI exceeded the international cut-off value for overweight, 17.92 kg/m\(^2\) for boys, and 17.75 kg/m\(^2\) for girls. This outcome was reported as point prevalence at 7 years.

**Wickens et al. (2013):** The outcome considered was ‘Overweight, number (%)’ and was defined with gender and age-specific cut-off values. This outcome was reported as point prevalence at 6 years.

The studies were statistically largely homogeneous ($\chi^2=0.70$, $P=0.4$, $\Gamma^2=0\%$). The result of meta-analysis did not show an association between maternal intake of probiotics during pregnancy and being overweight in the offspring (RR=0.76, 95% CI=0.45-1.27, 485 children).

**Figure 4.15. Forest plot of probiotics vs. placebo for overweight**

Measure: Binary: risk ratio
Heterogeneity: $Q = 0.709; \ df = 1; \ p = 0.4; \ I$-squared = 0%; tau-squared = 0.
Random effects model: 0.76 (0.457, 1.27)
4.5.9.6. Reported outcomes of obesity in the study by Louto 2010 at 10 years follow-up

This study followed up children until 10 years of age, reporting the adjusted mean (95% CI) difference of BMI, obesity and overweight as well as overall comparison of these adiposity measures across 2-10 years of follow-ups. There were no statistically significant differences for obesity measures between the groups either at 10 years or overall comparisons. The results are presented in the below table.

Table 4.7. List of the reported obesity outcomes in Louto et al. (2010) study at 10 years follow-up

<table>
<thead>
<tr>
<th>Outcome: Overweight</th>
<th>Probiotic n/N</th>
<th>Placebo n/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luoto</td>
<td>11/54</td>
<td>16/59</td>
</tr>
<tr>
<td>Wickens</td>
<td>17/245</td>
<td>9/127</td>
</tr>
<tr>
<td>Subtotal</td>
<td>299</td>
<td>186</td>
</tr>
</tbody>
</table>

4.5.10. Discussion of the evidence synthesis of maternal probiotic consumption during pregnancy and prevention of obesity in the offspring

4.5.10.1. Summary of main results

This systematic review summarised data from four RCTs of prenatal intake of probiotics for the prevention of childhood adiposity including a total of 1,610 children with follow-up ranging from 6.5 to 10 years (mean 7 years). One study was rated unclear for the method of allocation concealment (25%) and performance bias was judged as high risk in two studies (50%). Two studies also were assessed as unclear (25%) and high risk (25%) respectively for the detection bias domain. In addition,
half of the studies (50%) were deemed as high risk for loss to follow-up. The findings from this systematic review and meta-analysis do not provide evidence that prenatal intake of probiotics protects against developing childhood obesity. These results, however, need to be considered with caution due to the risk of bias in included trials, the limited number of studies and the small sample size in most studies.

It is worth noting that with the exception of one study conducted by Luoto et al., (2010), the others were primarily designed to assess outcomes other than adiposity in childhood, and reported growth measures as their secondary outcomes in further follow-up assessments. In the study by Luoto and colleagues (2010), women in both study arms also received intensive nutritional counseling complying with current recommendations for healthy fat and fibre content. Despite this, there was not a significant difference between the probiotic and placebo groups for the adiposity measures at any of the follow-ups.

**4.5.10.2. Overall completeness and applicability of evidence**

No evidence was found that prenatal intake of probiotic is effective for the prevention of adiposity in the offspring. The heterogeneity between the trials limited the findings and random effect models were used to pool the results. Heterogeneity between studies originated from varied types and dosage of probiotics, sample size, duration of intervention, different methods for reporting the outcome measures across studies and diverse locations/settings. Three studies used mixed strains of probiotics and the other a single probiotic strain. Due to the relatively small number of trials, it was not possible to test for differences originating from the type, dosage and duration of intervention. Moreover, attrition bias was an issue in two trials and one study used imputed analysis.

**4.5.10.3. Quality of evidence**

Overall, the trials were at moderate to high risk of bias, summary of risk of bias assessment in probiotic studies (Figure 4.10). A large proportion of studies rated as unclear on many risk of bias domains and some having a high risk of bias for individual quality domains. High loss to follow-up was a leading concern in two of the included trials (50%). Also the number of studies and participants contributed in meta-analyses might downgrade the quality of the evidence.
4.5.10.4. Strength and weakness of this systematic review for probiotic consumption during pregnancy

The main strengths and weaknesses of the current systematic review are discussed in section 4.5.6.4.

4.5.10.5. Agreement and disagreement with other reviews

This is the first systematic review and meta-analysis undertaken to examine the efficacy of prenatal consumption of probiotics for prevention of the long-term adiposity in children.

4.6.10.6. Author’s conclusion

Implications for practice

The results of the current systematic review do not support the hypothesis that supplementation of pregnant women with probiotics could prevent obesity in the offspring. Given the high heterogeneity between studies and attrition bias, along with small number of studies, the efficacy of probiotics intake during pregnancy for prevention of childhood adiposity in long-term remains unclear.

Implications for research

Taking the volume and quality of the available evidence into account, there is insufficient evidence as to whether prenatal probiotic intake could prevent the development of obesity in children. The efficacy of the intervention on childhood obesity needs to be addressed in further, large and high quality RCTs with coherent methods. Given that only one of the studies included had been established to explicitly examine the effect of prenatal consumption of probiotics on obesity in offspring, such RCTs need to be dedicated trials designed specifically to examine this question. Combined interventions of maintaining balanced nutrition and probiotic supplementation during pregnancy will also provide more validated means for the efficacy of probiotics, particularly in obese women who are at high risk of developing GDM.

The type of probiotic’ strain as well as their required dosage are key elements for consideration in future trials. Such studies should also consider measurement of obesity in children using a number of techniques including anthropometric measurements as well as adipose distribution such as fat mass, SFT. There is also a
need for well-designed epidemiological studies from under-reported populations, since the current evidence originates dominantly from the developed regions.
4.5.11. Description of included studies of maternal LG index diet during pregnancy and prevention of obesity in the offspring

A total of two included studies examined the impact of LG index diet during pregnancy on the development of obesity in offspring (including a total of 338 children). The characteristics and study population of these trials are shown in Table 4.8. The studies were conducted in Australia and Ireland.

The study conducted by Horan (Horan et al., 2016) had a large sample size of 800 women at recruitment and the study by Louie (Louie, Markovic, Ross, Foote, & Brand-Miller, 2015) involved 99 pregnant women at randomisation. One trial followed up the children up to 3 months of age (Louie et al., 2015) and the study by Horan et al. (2016) completed the follow-up by 6 months of age. The studies included women with a diagnosis of gestational diabetes mellitus (Louie et al., 2015) and women with a history of macrosomic baby (Horan et al., 2016).

There were substantial differences in the reported outcomes of the two studies. The study conducted by Louie et al. (2015) reported only the adjusted measures of weight and length in children, whereas a variety of anthropometric outcome measures were reported by Horan et al. (2016) study.
Table 4.8. Characteristics of the included trials and study population of LG index diet and prevention of obesity

<table>
<thead>
<tr>
<th>Primary article</th>
<th>Companion articles</th>
<th>Country, enrolment period</th>
<th>Trial type</th>
<th>Name of the study arms</th>
<th>No. of participants</th>
<th>No. at last F-U**</th>
<th>Time points measured</th>
<th>Age at last F-U</th>
<th>Sample: high risk or low risk</th>
<th>Outcomes reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Louie, et al., 2015)</td>
<td>(Kizirian et al., 2013; Louie et al., 2011; Markovic† et al., 2016)</td>
<td>Australia 2008-10</td>
<td>RCT</td>
<td>LG index &amp; (HF) High-fibre moderate-GI diet</td>
<td>99 mothers</td>
<td>58: 33 vs. 25</td>
<td>Birth &amp; 3 months</td>
<td>3 months</td>
<td>Diagnosed with GDM#</td>
<td>-Weight for age percentile (kg) -Length for age percentile (cm)</td>
</tr>
<tr>
<td>(Horan et al., 2016)</td>
<td>(Donnelly, Walsh, Byrne, Molloy, &amp; McAuliffe, 2015; Horan, McGowan, Gibney, Donnelly, &amp; McAuliffe, 2014; McGowan, Walsh, Byrne, Curran, &amp; McAuliffe, 2013; Walsh, McGowan, Mahony, Foley, &amp; McAuliffe, 2012; Walsh, Mahony, Foley, &amp; Mc McAuliffe, 2010)</td>
<td>Ireland 2007-2011</td>
<td>RCT</td>
<td>LG index &amp; usual antenatal care</td>
<td>800 mothers</td>
<td>280: 138 vs. 142</td>
<td>Birth &amp; 6 months</td>
<td>6 months</td>
<td>History of macrosomic baby</td>
<td>-Weight (kg) -Weight-for-length z-score -Length (cm) -Length-for-age z-score -BMI z-score -Sum of SFT -Mid-upper-arm circum.# -Triceps skinfold-for-age z-score -Subscapular skinfold-for-age z-score -Abdominal circum. -Chest circum. -Hip circum. -Thigh circum. -Biceps skinfold -Thigh skinfold -Sum triceps &amp; subscapular skinfolds -Triceps: subscapular skinfold ratio -Waist: Hip circum. ratio -Hip circum. length ratio</td>
</tr>
</tbody>
</table>

*Published data & conference presentations, no unique data extracted from conference abstracts
**Follow-up
***only birth outcomes reported in 2016 paper
#Gestational Diabetes Mellitus
†Indicates the No. at randomisation, where recruitment has occurred prenataally
#Circumference

224
The characteristics of LG index diet interventions and the control groups in the included trials are presented in Table 4.9. Comparable baseline characteristics have been reported in the included trials. Both included studies applied the intervention only throughout pregnancy, from the third (Louie et al., 2015) and second trimester of pregnancy (Horan et al., 2016).

In the study conducted by Louie et al. (2015), women attended at least three face-to-face visits with the study dietician, scheduled to coincide with regular antenatal visits, and were advised to have a LG index target of ≤50. The intervention in Horan et al. (2016) study involved a dietary education session lasting 2hrs, in groups of 2-6 women with the research dietician. Women were advised on: a) general healthy eating guidelines for pregnancy, following the food pyramid; b) the definition, concept, and rationale on GI for use in pregnancy. Women were encouraged to choose as many low GI foods as possible and to exchange high GI carbohydrates for low GI alternatives and received written resources about low GI foods. The recommended low GI diet was caloric balance, meaning women were not advised to reduce their total caloric intake but the source of calorie in their diet. The research dietician met with the patients at 28 and 34 weeks’ gestation for reinforcement of the low GI diet and to answer any dietary queries.

In the trial by Louie et al. (2015) women were supplemented in both study arms, as appropriate by the treating endocrinologist, by iron and iodine since the study diet could provide all essential nutrients other than the two minerals. Women in the study conducted by Horan et al. (2016) did not receive any supplementation.

The Louie et al. (2015) trial assessed dietary compliance during the intervention period, every 2–3 weeks with an approval from multiple-pass 24-h recalls, and where the subject’s diet deviated from the assigned diet, they were encouraged to choose more foods that conformed to their assigned diet. A 3-day food record was also completed at the end of the intervention and its results showed a six-unit difference in dietary GI index between the two groups (intervention vs. control: 47/53; P < 0.001). The Horan et al. (2016) study assessed the adherence to the LG index diet, at the 34 weeks antenatal visit, using a five point Likert-type scale (1=“I followed the recommended diet all of the time”; 5=“I followed the recommended diet none of the
time”). In addition, acceptability of the diet was assessed using a 6-item questionnaire and 68% of women generally or strongly agreed that the diet was easy to follow. Women also completed 3-day food diaries in each trimester of pregnancy to define their glycemic index and glycemic load and at the third trimester, a lower glycemic index [56 (3.8) vs. 57.7 (3.9)] as well as glycemic load [127.1 (30.4) vs. 139.9 (37.5)] was observed in the intervention group compared to the control women.
### Table 4.9. Characteristics of LG index diet interventions in the included trials for prevention of obesity

<table>
<thead>
<tr>
<th>Primary article</th>
<th>Comparable baseline characteristic</th>
<th>Participants receiving intervention</th>
<th>Timing of Intervention in pregnancy</th>
<th>Intake of intervention From/until*</th>
<th>Duration of intervention (months)**</th>
<th>Type of food intervention</th>
<th>Control arm</th>
<th>Substitutes prescribed (in both arms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Louie et al., 2015</td>
<td>Yes (At baseline, subjects in the LGI group had significantly higher 2hrs post-load blood glucose levels)</td>
<td>Prenatally only</td>
<td>29wks.</td>
<td>29wks. to delivery</td>
<td>2 months &amp; 3wks</td>
<td>Healthy diet containing: protein (15–25%), fat (25–30%), carbohydrate (40–45%) &amp; an LGI target ≤50</td>
<td>Diet with high-fibre content &amp; moderate GI</td>
<td>Iron and iodine Supplemented as appropriate by the treating endocrinologist</td>
</tr>
<tr>
<td>Horan et al., 2016</td>
<td>Yes</td>
<td>Prenatally only</td>
<td>14wks.</td>
<td>14wks. to delivery</td>
<td>6-6.5</td>
<td>LG index diet under dietetic supervision</td>
<td>No dietary intervention</td>
<td>-</td>
</tr>
</tbody>
</table>

* Mothers have continued food avoidance at different time points e.g. Late start of solids

** Indicates total duration in pregnancy plus after birth, if applicable
4.5.12. Risk of bias in studies of maternal LG index diet during pregnancy and prevention of obesity in the offspring

The summary of risk of bias of trials on LG index diet is presented in Figure 4.16. The reviewer’s judgment for the risk of bias assessment of LG index diet studies is shown in appendix 4.7.
### Summary of Risk of Bias Assessment

<table>
<thead>
<tr>
<th>Short Title</th>
<th>Random Sequence Generation</th>
<th>Allocation Concealment</th>
<th>Double Blinding</th>
<th>Blinding of Outcome Assessment</th>
<th>Incomplete Outcome Data</th>
<th>Selective Outcome Reporting</th>
<th>Other Sources of Bias</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horan 2016</td>
<td>+</td>
<td>?</td>
<td>-</td>
<td>?</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

**Figure 4.16.** Summary of risk of bias assessment in the included trials of LG index diet and prevention of obesity in the offspring.
4.5.12.1. Random sequence generation (selection bias)
Both studies were rated as having a low likelihood of selection bias as they randomised the participants by using a computer system.

4.5.12.2. Allocation concealment
One study failed to report the method of allocation (Louie et al., 2015) and so was rated as having an unclear risk for allocation concealment. In the study by Horan et al. (2016) the research staff were involved in the randomisation process, using opaque sealed envelopes and was rated as having unclear risk of bias.

4.5.12.3. Double blinding (performance bias)
The study conducted by Horan et al. (2016) was rated as high risk of bias since it is stated that the staff and obstetricians were blind to the intervention although the participants were aware of the type of intervention. The study by Louie and colleagues (2015) was classified as having an unclear risk of bias, since it did not provide any information on blinding of the research staff or the participants.

4.5.12.4. Blinding of outcome assessment (detection bias)
The two included studies were rated as unclear risk of bias since they did not give an indication whether the measurements were conducted in a blinded fashion.

4.5.12.5. Incomplete outcome data (attrition bias)
A high rate of loss to follow-up was observed in the included studies and both failed to report the reasons for missing and were therefore rated as high risk.

4.5.12.6. Selective outcome reporting (reporting bias)
In both included trials, the pre-specified outcomes of obesity were reported; thus the trials were rated as low risk for selective outcome reporting.

4.5.12.7. Other potential sources of bias
Other sources of bias in both the included trials were considered and one study was assessed as high risk (Horan et al., 2016), as no detailed dietary data was collected at 6 months postpartum. Therefore, there is no information regarding whether women continued the low GI diet or had reverted to pre-pregnancy dietary habits. The study by Louie et al. (2015) was rated as unclear risk, since women were encouraged to
continue their assigned diet after delivery; however the post-partum diet was not formally assessed and women may have reverted to their habitual eating pattern.

4.5.13. Meta-analyses of effectiveness of maternal LG index diet during pregnancy and prevention of obesity in the offspring

The type and method of measuring the obesity-related outcomes in the two included studies for LG index diet were not similar. Therefore it was not possible to perform meta-analysis and the reported outcomes of interest for the current systematic review are described narratively in the following sections.

4.5.13.1. Narrative description of the results in the Louie et al. (2015) study

The study of Louie et al. (2015) reported adjusted adiposity-related measures in offspring by 3 months of age, listed in the table (4.10). The percentiles of the outcomes measured (weight for age, length for age and weight for length) were based on the Centers for Disease Control and Prevention growth charts and the reported outcomes were also adjusted for breast-feeding status (exclusive vs. non-exclusive). The outcome reported (weight gain per day) was adjusted for both breast-feeding status and gender. The results of this study indicated that LG index diet in pregnant women in comparison to a diet high in fibre did not have an influence on the obesity-related outcomes in children.

Table 4.10. List of the reported obesity outcomes in Louie et al. (2015) study

<table>
<thead>
<tr>
<th></th>
<th>LG index (n=31) Mean (95% CI)</th>
<th>High-Fibre (n=21) Mean (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight for age percentile</td>
<td>69.6 (60.5–78.8)</td>
<td>68.0 (56.9–79.1)</td>
<td>0.72</td>
</tr>
<tr>
<td>Length for age percentile</td>
<td>47.9 (38.6–57.2)</td>
<td>48.1 (36.9–59.3)</td>
<td>0.97</td>
</tr>
<tr>
<td>Weight for length percentile</td>
<td>72.4 (61.2–83.6)</td>
<td>64.6 (51.0–78.1)</td>
<td>0.51</td>
</tr>
<tr>
<td>Weight gain per day (g)</td>
<td>32.6 (29.9–35.4)</td>
<td>31.4 (27.5–35.3)</td>
<td>0.52</td>
</tr>
</tbody>
</table>

4.5.13.2. Narrative description of the results in the Horan et al. (2016) study

Horan et al. (2016) reported a range of adiposity-related measures in offspring by 6 months of age, listed in the below table. WHO growth standards were used to convert the infant measurements to z-scores, which are adjusted for infant age and gender. At 6 months, there were no differences in any infant anthropometric measures between the control (routine care) and intervention (LG index diet) groups (Table 4.11).
This study however, showed that maternal GI index in third trimester of pregnancy was positively associated with triceps skinfold thickness for age-z-score and biceps skinfold thickness at 6 months (p=0.003).

4.5.14. Discussion of the evidence synthesis of maternal LG index diet during pregnancy and prevention of obesity in the offspring

4.5.14.1 Summary of main results
This systematic review summarised data from two RCTs that evaluated the efficacy of a LG index diet during pregnancy on adiposity outcomes in children. The review included a total of 338 children with the follow-up duration from three to six months (mean 4.5 months). Allocation concealment was rated as unclear in both studies and detection bias was judged as either unclear or high in each of the studies. Furthermore, there was a high rate of loss to follow-up in both studies. It was not possible to conduct any meta-analyses due to the various outcomes reported in these studies and therefore, the findings were described narratively. Neither study provides evidence for a protective association between LG index diet during pregnancy and subsequent development of childhood adiposity. It is worth mentioning that both studies had a high risk of bias, particularly for loss to follow-up domain.

4.5.14.2. Overall completeness and applicability of evidence
It is important to note that only two RCTs were included in this systematic review and there was a low quality of evidence from individual studies that LG index diet during pregnancy is effective for the prevention of adiposity in the offspring. There...
was a high heterogeneity between the two trials in terms of timing of intervention, different controls and varied reported outcomes. It was not possible to conduct any meta-analyses due to the varied adiposity outcomes reported in the studies. In both studies, an insufficient number of women were followed up. The study by Horan et al., (2016) reported that only 35% of women (280 out of 800 recruited) returned for the follow-up assessment at 6 months. Louie and colleagues (2015) enrolled a smaller sample and 59% of women originally recruited (58 out of 99) were approached for the follow-up assessment at three months.

4.5.14.3. Quality of evidence
Overall, the trials were at high risk of bias, for the summary of risk of bias assessment (Figure 4.16). Both studies were rated as either unclear or high risk of bias for most individual quality domains and attrition bias was also a main issue in both studies.

4.5.14.4. Strength and weakness of this systematic review for LG index diet during pregnancy
The main strengths and weaknesses of the current systematic review are discussed in section 4.5.6.4. It is important to add that, due to the small number of LG index diet intervention studies that met the inclusion criteria for this systematic review and also inconsistencies between the reported outcomes, it was not possible to perform meta-analysis and data were described narratively.

4.5.14.5. Agreement and disagreement with other reviews
This is the first systematic review of RCTs that narratively described the efficacy of applying a LG index diet during pregnancy for the prevention of childhood obesity in the long-term.

4.6.14.6. Author’s conclusion
Implications for practice
The available evidence does not support the hypothesis that LG index diet in pregnant women can protect against developing childhood adiposity. Since only two studies fulfilled the inclusion criteria and the observed variability between the reported outcomes did not warrant meta-analyses, it remains an open question whether LG index diet during pregnancy would prevent developing childhood obesity in the long-term.


**Implications for research**

The quantity and quality of the current evidence indicates that the efficacy of LG index, as a prenatal dietary strategy, needs to be examined in further large well-designed RCTs. In addition, more consistent approaches are required for reporting the adiposity outcomes using combined methods of anthropometric and fat distribution measurements.

The optimal timing of LG index diet intervention is additionally an important element that needs to be addressed in future trials, since intervention as early as pre-pregnancy could deter either the extra or accelerated weight gain in pregnant women, particularly in those who are already obese or at high risk of developing GDM. The choice of control is also an important factor in interventional studies of this nature since it is generally hard to control in a double blind manner, and could potentially bias assessment of outcomes in the study arms. A practical approach could be to introduce a representative sample of pregnant women from the general population as the control or allocate independent participating centres for recruiting women into the intervention and control arms of the study.

Maintaining compliance with the recommended diet during pregnancy is clearly a major challenge. Food diaries provide the best subjective estimate of regular intake of food items, however participants may inaccurately report or inadvertently change their dietary habits while keeping a dietary record. Evidence from studies on LG index diet in pregnant women has shown that women had poor adherence to dietary change post-partum and reverted to their pre-intervention dietary intake (Fehler, Kennedy, & McCargar, 2007; Moses, Luebke, Petocz, & Brand-Miller, 2007; Stage, Ronneby, & Damm, 2004). Therefore, measuring of adherence to intervention using more rigorous methods such as biochemical parameters is recommended in future studies. More importantly, high attrition bias in both studies included in this review suggests that their results could have been biased by an unrepresentative sample of women originally recruited and thus the real efficacy of the intervention might have been underestimated. In both trials, women in the intervention arm had a significant lower glycemic index compared to the control group by third trimester of pregnancy, denoting the efficacy of intervention and in the mean time, adds weight to the importance of reducing loss to follow-up. Therefore, conducting longitudinal studies
prior to or as early as possible in pregnancy, with adequate sample size, is required to provide strong evidence with which to determine the effect of LG index diet during pregnancy on childhood obesity.
4.5.15. Description of included studies of maternal life-style change during pregnancy and prevention of obesity in the offspring

Two studies were grouped as lifestyle change, including a total of 375 children. The characteristics of the included trials, their companion papers and study population are presented in Table 4.12.

The included studies recruited a sample of 360 (Tanvig et al., 2015) and 250 (Rauh, Günther, Kunath, Stecher, & Hauner, 2015) of pregnant women at randomisation. The duration of follow-up were reported by 2.5-3 years (Tanvig et al., 2015) and 10-12 months (Rauh, et al., 2015). The studied samples were selected of obese women (Tanvig et al., 2015) and unselected women (Rauh, et al., 2015). The study conducted by Tanvig et al. (2015) also included an external reference group of lean mothers and their offspring for comparisons of offspring from their study arms with this group.

Great differences were observed between the studies for the reported obesity outcomes in children. A range of obesity outcomes were reported in the study conducted by Tanvig et al. (2015), whereas Rauh and colleagues (2015) only reported weight as the outcome in children.
Table 4.12. Characteristics of the included trials and study population of life-style change interventions and prevention of obesity

<table>
<thead>
<tr>
<th>Primary article</th>
<th>Companion articles</th>
<th>Country, enrolment period</th>
<th>Trial type</th>
<th>Name of the study arms</th>
<th>No. of participants</th>
<th>No. at last F-U**</th>
<th>Time points measured</th>
<th>Age at last F-U</th>
<th>Sample: high risk or low risk</th>
<th>Outcomes reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Tanvig et al., 2015)</td>
<td>(Tanvig, 2014; Tanvig et al., 2014)</td>
<td>Denmark 2007-10</td>
<td>RCT</td>
<td>Life style Int. &amp; control (routine care)</td>
<td>360 mothers</td>
<td>150: 77 vs. 73</td>
<td>Birth &amp; 2.5-3yrs.</td>
<td>2.5-3yrs.</td>
<td>Obese women</td>
<td>-Weight (Kg)</td>
</tr>
</tbody>
</table>

*Published data and conference presentations, no unique data were extracted from conference abstracts
**Randomised Controlled Trial
***Follow-up

Indicates the No. at randomisation, where recruitment has occurred prenatally
Table 4.13 shows the details of lifestyle change interventions and control groups applied in the included trials. Comparable baseline characteristics have been reported in the trial conducted by Tanvig et al. (2015); although in the study conducted by Rauh et al. (2015) a higher proportion of overweight and obese women were reported in the control group (31% vs. 16%) which resulted in a significantly higher self-reported weight and BMI before pregnancy in the control group compared to the intervention group.

The lifestyle intervention in the Tanvig et al. (2015) trial consisted of dietary counseling and physical activity. The dietary history, weight and level of activity of women in the intervention group were evaluated and then they received four individual dietary counselling sessions by trained dieticians aiming to limit their gestational weight gain by 5kg. The physical activity plan encouraged women to be physically active for 30-60 minutes daily and also a closed aerobics class was arranged with a physiotherapist for an hour a week. Women also participated in group sessions 4-6 times during pregnancy along with the physiotherapist, where using the inspired coaching methods, the aim was to improve women’s integration of physical activity in pregnancy and daily life. In addition, women were provided with a free, fulltime membership to a fitness centre with access to all types of aerobic classes and weight training. The intervention in the study conducted by Rauh et al. (2015) involved two individual counselling sessions by trained researchers at the 20th and 30th gestational week comprising of three components: healthy diet, advice on physical activity and weight monitoring using IOM recommendations for GWG.

The study conducted by Tanvig et al. (2015) assessed compliance with both the dietary counselling sessions and physical component by participation rate. Women were also asked whether participation in the study resulted in more healthy eating habits. A good compliance rate was reported where 92% of women attended all their dietary counseling sessions with 98% completing at least three sessions and 85% also believed that the study enhanced their dietary habits. Compliance with the physical component of the intervention was reported as 10.4 hours for attending the 20 aerobic classes and 78% also undertook leisure time sporting activities along with the aerobic classes. At the same time, 21% of women in the control group reported having
healthier eating habits following participation in the study and, 65% reported some type of sporting leisure activities.

The Rauh et al. (2015) trial measured compliance with the nutritional counselling by assessing dietary intake during pregnancy using 7-day dietary records, at 16-18 (baseline), 26-28 and 36-38 weeks of gestation in the intervention group. The dietary intake of women in the control group was also measured at baseline and third trimester of pregnancy. Level of physical activity was assessed in women of both groups by the long version of International Physical Activity Questionnaire (IPAQ) (Carig, et al., 2003) at the three time points in pregnancy as above. The study did not report how the dietary pattern and level of physical activity differed between the two study groups at the end of the intervention.
Table 4.13. Characteristics of life-style change interventions in the included trials for prevention of obesity

<table>
<thead>
<tr>
<th>Primary article</th>
<th>Comparable baseline characteristic</th>
<th>Participants receiving intervention</th>
<th>Timing of Intervention in pregnancy</th>
<th>Intake of intervention From/until*</th>
<th>Duration of intervention (months)**</th>
<th>Type of nutrition intervention</th>
<th>Control arm</th>
<th>Substitutes prescribed (in both arms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tanvig et al., 2015</td>
<td>Yes</td>
<td>Prenatally only</td>
<td>10-14wks</td>
<td>10-14wks to delivery</td>
<td>6-7.5</td>
<td>Dietary counseling+ Physical activity</td>
<td>Routine obstetric care</td>
<td>-</td>
</tr>
<tr>
<td>Rauh et al., 2015</td>
<td>No, Higher weight and BMI, before pregnancy was reported in the control group</td>
<td>Prenatally only</td>
<td>At 20th &amp; 30th gestational wks.</td>
<td>Individual counseling at 20th &amp; 30th wks.</td>
<td>2.5-5</td>
<td>Individual nutrition counseling+ physical activity+ weight monitoring</td>
<td>Routine prenatal care</td>
<td>-</td>
</tr>
</tbody>
</table>

*Indicates total duration in pregnancy plus after birth, if applicable
4.5.16. Risk of bias in studies of maternal life-style change during pregnancy and prevention of obesity in the offspring

The summary of risk of bias of trials on life-style change is presented in Figure 4.17. The reviewer’s judgment for the risk of bias assessment of life-style change studies is shown in appendix 4.8.
<table>
<thead>
<tr>
<th>Short Title</th>
<th>Random Sequence Generation</th>
<th>Allocation Concealment</th>
<th>Double Blinding</th>
<th>Blinding of Outcome Assessment</th>
<th>Incomplete Outcome Data</th>
<th>Selective Outcome Reporting</th>
<th>Other Sources of Bias</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rauh 2015</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>?</td>
</tr>
<tr>
<td>Tanvig (2015)</td>
<td>+</td>
<td>?</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>?</td>
</tr>
</tbody>
</table>

Figure 4.17. Summary of risk of bias assessment in the included trials of life-style change and prevention of obesity in the offspring
4.5.16.1. Random sequence generation (selection bias)
Both studies used a computer-generated randomisation table and were, therefore, rated as low risk of bias. It is worth adding that randomisation in Rauh et al. (2015) trial was conducted at cluster level rather than individuals i.e. gynaecological practices.

4.5.16.2. Allocation concealment
In the study by Rauh et al. (2015), a researcher that was not involved in the study design performed the randomisation and the study was rated as having a low risk of bias for allocation concealment. The study by Tanvig et al. (2015) was rated as having an unclear risk of bias since the study involved a doctor and research midwife in enrolment and women themselves picked up their randomisation number from a basket, with no detail how the numbers were presented.

4.5.16.3. Double blinding (performance bias)
In both of the included studies, neither the study staff nor the participants were blinded to the study intervention and studies were rated as high risk of bias.

4.5.16.4. Blinding of outcome assessment (detection bias)
The Rauh et al. (2015) trial was open label due to the nature of the intervention and was rated as high risk. In the study by Tanvig et al. (2015), the outcome assessor was blinded to the RCT intervention and was rated as low for detection bias.

4.5.16.5. Incomplete outcome data (attrition bias)
Both studies were rated as having a high risk of attrition bias. In the Rauh et al. (2015) study, due to the higher participation rate, there was an unequal group size between the intervention and control groups at recruitment with the number of participants doubled in the intervention arm (83 vs. 167). A higher attrition bias was observed for the control group at the follow-up assessment where 78% and 88% (65 vs. 148) of women were approached in the standard care and intervention groups respectively. The trial by Tanvig et al. (2015) approached less than 50% of women in the follow-up assessment and reason(s) for attrition are not stated.

4.5.16.6. Selective outcome reporting (reporting bias)
The pre-specified outcomes are reported in both studies and thus rated as low risk of bias.
4.5.17. Meta-analyses of effectiveness of maternal life-style change during pregnancy and prevention of obesity in the offspring

Only one outcome, defined as “weight” was reported in common in the two included studies. A meta-analysis for the outcome measure as “weight” yielded a great level of heterogeneity between the included studies ($\chi^2=6.55$, $p=0.01$, $I^2=84.7\%$) (Forest plot is not shown). Therefore, the results from these studies are reported narratively in the following sections.

4.5.17.1. Narrative description of the results of the Tanvig et al. (2015) study

The study of Tanvig et al. (2015) reported a range of adiposity-related outcomes in offspring by 2.8 years, listed in the table below (4.14). Weight and height (to the nearest 0.1) were measured using a digital weight and portable stadiometer respectively. A Harpenden skinfold calliper was used to measure triceps and subscapular skinfold thickness to the nearest 0.1 mm. BMI-Z was reported based on age and sex-specific Danish standards. Overweight and obesity were classified as one outcome due to the small number of children and were identified using the IOTF criteria. The results showed that lifestyle intervention, including dietary advice, coaching and exercise during pregnancy compared to routine obstetric care did not have an influence on the obesity-related measures in offspring.

Table 4.14. List of the reported obesity outcomes in Tanvig et al. (2015) study

<table>
<thead>
<tr>
<th></th>
<th>Lifestyle change (n=82) Mean (SD)</th>
<th>Routine obstetric care (n=75) Mean (SD)</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>14.7±1.64</td>
<td>14.4±1.63</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>94.6±3.42</td>
<td>94.6±3.52</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>BMI</td>
<td>16.4±1.3</td>
<td>16.1±1.2</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>BMI-Z</td>
<td>0.06±1.05</td>
<td>-0.18±1.70</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Overweight/obese (No.)</td>
<td>9 (10.9%)</td>
<td>5 (6.7%)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Triceps skinfold thickness</td>
<td>8.3±1.68</td>
<td>8.3±1.96</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Subscapular skinfold thickness</td>
<td>6.1±1.58</td>
<td>6±1.09</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>
4.5.17.2. Narrative description of the results of the Rauh et al. (2015) study
The study conducted by Rauh et al. (2015) only reported weight in the offspring, as the outcome of interest for the current systematic review, measured at different time points up to 10-12 months of age. Women were asked for the weight development of their children as assessed by the paediatricians at the routine check-ups. The results of the unadjusted analysis showed a significant association between the weight of children, between 10-12 months, as infants born to mothers in the intervention group weighed less than control group infants (9.382±0.93 vs. 9.736±0.99, p=0.01). However the effect disappeared in an adjusted analysis for practice (random factor), maternal age, pre-pregnancy BMI, infant age at follow-up and birth weight (p=0.099). Also, adjusting the model for an added variable as “breastfeeding duration” did not show a statistical significant difference between the two groups (p=0.14).

4.5.18. Discussion of the evidence synthesis of maternal life-style change during pregnancy and prevention of obesity in the offspring

4.5.18.1. Summary of main results
This systematic review summarised data from two RCTs that evaluated the efficacy of a life-style change during pregnancy on adiposity outcomes in children. The review included a total number of 375 children with follow-up duration ranging from 10-12 months to 2.5-3 years (mean 20-24 months). Method of allocation concealment was rated as low (50%) and unclear (50%) in the included studies. In both studies, staff and participants were aware of the allocation assignment and additionally, detection bias was judged as high in one study. High rate of loss to follow-up was reported in the included studies. Substantial statistical heterogeneity was observed in the meta-analysis conducted for “weight” as the only outcome reported in common in both studies. It was not possible to conduct any other meta-analyses due to inconsistency between the reported outcomes and, therefore, the findings were described narratively. Findings from each individual study do not provide evidence that life-style change during pregnancy, consisting of dietary counselling and physical activity components, could be beneficial in subsequent development of childhood adiposity.

4.5.18.2. Overall completeness and applicability of evidence
It is important to note that only two RCTS were included in this systematic review and there was a low quality of evidence from individual studies that life-style change
during pregnancy could be beneficial for prevention of adiposity in the offspring. There was a high heterogeneity between the two trials in terms of the study sample (obese vs. unselected), timing and duration of intervention, duration of follow-up, using varied methods for measurement of obesity outcomes and diverse reported outcomes as well as measurement of compliance with the intervention. The only conducted meta-analysis showed a high statistical heterogeneity between studies and it was not possible to conduct any other meta-analyses because of the diverse adiposity outcomes reported in studies. High loss to follow-up was also a concern in the studies. In the study conducted by Tanvig and colleagues (2015), less than 50% of women were included in the follow up assessment. The study by Rauh et al. (2015) approached 88% of women in the intervention group but 78% in the control. Since the number of participants in the control group was half of the intervention group at recruitment, the lower rate of follow-up in the control arm was a major problem. A higher proportion of women in the control group of this study, with a significant difference (31% vs. 16%), also tended to be obese at recruitment.

4.5.18.3. Quality of evidence
Overall, the trials were at high risk of bias, as shown in Figure 4.17 for the summary of risk of bias assessment. Both studies were mainly rated as high risk of bias for most individual quality domains.

4.5.18.4. Strength and weakness of this systematic review for life-style change during pregnancy
The main strengths and weaknesses of the current systematic review are discussed in section 4.5.6.4. However, specifically in relation to the life-style change as an intervention, it is important to add high statistical heterogeneity was observed in the one meta-analysis conducted and because of the inconsistencies between the reported outcomes in studies, it was not possible to perform any more meta-analysis and data were described narratively.

4.5.18.5. Agreement and disagreement with other reviews
This is the first systematic review of RCTs to assess the efficacy of life-style change intervention during pregnancy, using a narrative approach, for the prevention of childhood obesity in the long-term.
4.6.18.6. Author’s conclusion

Implications for practice
Current data does not provide evidence that life-style change, as an intervention during pregnancy, can protect against developing childhood adiposity; however the evidence is not conclusive. There were a small number of studies included and the observed variability between reported outcomes did not allow for conducting detailed meta-analyses, so it remains uncertain whether life-style change in pregnancy would be a beneficial preventive approach against developing childhood obesity in the long-term.

Implications for research
The quantity and quality of the current evidence signifies that the efficacy of life-style change needs to be assessed in further large well-designed RCTs. In addition, more consistent approaches are required for reporting the adiposity outcomes using combined methods of anthropometric and fat distribution measurements.

The ideal timing of life-style change interventions is also an important area that needs to be precisely addressed in future studies. A recent study has flagged inappropriate timing of the life-style interventions during pregnancy i.e. late onset as the main concern that these trials have failed to control maternal, and more specifically foetal overgrowth (Catalano & DeMouzon, 2015). Catalano & deMouzon (2015) reasoned that since the metabolic conditioning programmes the placental function and gene expression in the first trimester of pregnancy, the interventions need to commence prior to conception to normalise metabolic conditioning in women and thus, to decrease the related complications in infants. Furthermore, blinding of staff and participants in studies of this nature is difficult and the fact that the participants were aware of their group allocation in the included studies in this review might have partly minimised the participation rate, particularly among the women in control group. Tanvig and colleagues (2015) also included an external reference group of lean mothers in their study, which could be a practical strategy in future trials.

Maintaining compliance with the recommended dietary advice as well as physical activity during pregnancy is a key issue and highlights the importance of maintaining women’s motivation throughout the intervention. More importantly, high attrition bias in the included studies suggests that their results could have been biased by an
unrepresentative sample of women and thus the real efficacy of life-style intervention might have been underscored. Significant research by conducting longitudinal studies with early onset, prior to pregnancy, and adequate sample size is required to provide strong evidence with which to determine the effect of life-style change during pregnancy on obesity in children. It is worth adding that reasons for non-participation in life-style change interventional studies have been investigated. Findings from one study in Norway showed that this group are usually content with their personal nutrition and fitness plan, they are younger, with a higher educational status and also smoke (Sagedal, 2014). In contrast, the results of another study in Denmark indicated that non-participants were at an educational level comparable to the participants, and tended to be younger, had lower parity, were more frequently non-smokers and either married or cohabiting with their partner (Gesche, Renault, Nørgaard, & Nilas, 2014). In both of these studies, participants and non-participant women had comparable BMI. These findings highlight the importance of cultural differences when designing community-based studies aiming to make behavioural modifications at the population level.

4.6. Round-up conclusion

The role of environmental factors in developing many chronic diseases is well-documented and early life nutritional interventions provide a window of opportunity for longer-term health in the offspring. The current body of evidence underscores the importance of dietary and life-style interventions during pregnancy for the prevention of childhood obesity. These findings, however, are not conclusive due to the small number of studies included in most intervention groups and also, great heterogeneity observed between the included studies. Well-designed high-quality studies, with early onset prior to pregnancy or the first trimester of pregnancy, need to address the hypothesised effect of nutritional interventions during pregnancy for prevention of adiposity in children. It is advised that nutritional trials should establish defined rules and objectives in their design such as measurement of basal nutrient status and the change following the intake of intervention to allow valid testing of the hypothesised association(s) (Heaney, 2014).
4.7. Description of included studies of maternal vitamin/micronutrient consumption during pregnancy and boosting growth standards in the offspring

Following the search strategy for this systematic review, a sub-group of studies were identified that supplemented pregnant women with vitamins/micronutrients. Further investigation of these studies revealed that these trials were all conducted in developing countries and were initially intended to enhance foetal birth weight rather than prevention of obesity-adiposity. Further follow-up assessments in these studies have also investigated the efficacy of the prenatal intervention on boosting childhood growth standards i.e. patterns of linear growth and body size. It was decided to only present the descriptive findings for this dietary group in the following sub-section, as the goal of these trials was originally different from the defined aim in these systematic reviews i.e. boosting growth standards rather than prevention of obesity, and risk of bias assessment and meta-analyses were not conducted.

Four studies were grouped as vitamins/micronutrient, including 3,040 children. The characteristics of the included trials, their companion papers and study population are presented in Table 4.15. Two studies were conducted in Nepal, one in Bangladesh and one in Gambia. Two studies were community-based involving cluster randomisation of villages including women of childbearing age (Hawkesworth, Prentice, Fulford, & Moore, 2008; Stewart, Christian, LeClerq, West, & Khatry, 2009).

The longest follow-up period was 11-17 years, in the study conducted by Hawksworth et al. (2008), and followed by 6-8 years in the study by Stewart et al. (2009). The shortest follow-up period was reported in the Roth et al. (2013) study. The largest study sample was reported in Stewart et al. (2009) with 4,998 mothers enrolled followed by Hawksworth et al. (2008) with 1,460 pregnant mothers at enrolment. The smallest sample size was observed in the study conducted by Roth et al. (2013) with 160 mothers randomised at recruitment.

Compliance with the treatment was assessed using a variety of methods as discrepancy estimate method (providing participants with a varying quantity),
monitoring and recording by research team staff and number of doses received divided by number of doses scheduled times 100.

The outcome measures in the studies by Hawksworth et al. (2008) and Roth et al. (2013) were presented by child gender only. All studies reported detailed anthropometric measures in children to assess as to whether the maternal intervention improved the body composition in children later in life.
Table 4.15. Characteristics of the included trials and study population of vitamins/micronutrients for improving growth measures in children

<table>
<thead>
<tr>
<th>Primary article</th>
<th>Companion articles¹</th>
<th>Country, enrolment period</th>
<th>Trial type</th>
<th>Name and No. of study arms</th>
<th>No. of participants</th>
<th>No. at last F-U***</th>
<th>Time points measured</th>
<th>Age at last F-U</th>
<th>Sample</th>
<th>Outcomes reported</th>
</tr>
</thead>
</table>
| (Vaidya et al., 2008) | (Osrin et al., 2005) | Nepal 2002-03 | RCT | Multiple micronutrient supplement vs. routine iron & folic acid supplements | 1,200 mothers | 917: 462 vs. 455 | Birth & 2.5yrs | 2.5yrs. | Unselected | -Head Circumference (cm)  
-Weight (Kg)  
-Height (cm)  
-Chest circumference (cm)  
-BMI (kg/m²)  
-Hip circumference (cm)  
-Waist circumference  
-Mid-upper-arm-circumference |
| (Hawkesworth, et al., 2008) | (Ceesay et al., 1997) | Gambia 1989-1994 | CRT | Protein-supplement energy & control | 1,460 women leading to 2047 singleton live births | 1,317: 630 vs. 687 children measured | Birth & 11-17yrs. | 11-17 yrs. | Undernourished women | -Height, cm  
-Weight, kg  
-BMI, kg/m²  
-Body fat  
-Trunk fat  
-Fat Mass Index (kg/m⁴)  
-Fat Free Mass Index (kg/m³) |
| (Stewart, et al., 2009) | (Christian et al., 2003) | Nepal 1998-2001 | CRT | 4 Int. arms as: Folic acid or Folic acid + iron or Folic acid +iron+zine or Multiple | 4,998 mothers | 658 vs. 674 vs. 708 vs. 749 vs. 735 | Birth & 6-8yrs. | 6-8yrs. | Community trial (from 30 villages) | -Weight (Kg)  
-Height (cm)  
-Arm circumference (cm)  
-Waist circumference (cm)  
-BMI (kg/m²) |
<table>
<thead>
<tr>
<th>Study (2013)</th>
<th>Country</th>
<th>Study</th>
<th>Intervention</th>
<th>Sample Size</th>
<th>Follow-up</th>
<th>Site</th>
<th>Measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Roth, Perumal, Al Mahmud, &amp; Baqui)</td>
<td>Bangladesh 2010-11</td>
<td>PC-RCT</td>
<td>Vitamin D &amp; Placebo</td>
<td>160 mothers</td>
<td>1yr</td>
<td>Unselected</td>
<td>-Subscapular [mm] -Sum 4 SFT [mm] -Triceps skinfold thickness -Subscapular skinfold thickness -Arm fat area -Arm muscle area -Height-for-age-z-score -Weight-for-age-z-score -BMI-for-age-z-score</td>
</tr>
</tbody>
</table>

*Published data and conference presentations, no unique data were extracted from conference abstracts*  
**Placebo-controlled Randomised Controlled Trial or Randomised Controlled Trial or Cluster Randomised Trial**  
***Indicates the number at randomisation, where recruitment has occurred prenatally***  
****Follow-up***
Table 4.16 shows the details of vitamin/micronutrient interventions and control applied in the included studies. Comparable characteristics have been reported in two trials. In the study by Stewart et al. (2009), small differences were observed in ethnic composition and land holding and women in the control group also weighed slightly less. The study by Roth et al (2013) reported mothers in the Vitamin D group had a higher level of education, although this difference was not statistically significant.

All studies applied the intervention during pregnancy only (Table 4.16). The longest duration of intervention was reported in the studies conducted by Vaidya et al. (2008 and Stewart et al. (2009) for 5-7 and 6.5-7 months respectively and the shortest follow-up period was 2.5-3 months (Roth et al., 2013). One study supplemented women with multiple micronutrients (Vaidya et al., 2008), whereas the study by Stewart et al. (2009) defined four different intervention arms including multiple micronutrients. The study by Hawksworth et al. (2008) used protein-supplement energy biscuits as the intervention and in the study by Roth et al. (2013) women in the intervention group were supplemented with vitamin D.

The nature of control varied between studies. The control group was defined as the routine care, as iron and folic acid, in the study by Vaidya et al. (2008), Vitamin A alone in Stewart et al. (2009) study, miglyol oil in the study by Roth et al. (2013) and no treatment in Hawksworth et al. (2008) study. Women in the control group in Hawksworth et al. (2008) received the protein-energy supplement for 20 weeks after delivery. Vitamin/supplement preparations included capsules and oil drops.
Table 4.16. Characteristics of vitamin/micronutrient interventions in the included trials for improving growth measures

<table>
<thead>
<tr>
<th>Primary article</th>
<th>Comparable baseline characteristic</th>
<th>Participants receiving intervention</th>
<th>Timing of intervention in pregnancy</th>
<th>Intake of intervention From/until</th>
<th>Duration of intervention (months)</th>
<th>Vitamin &amp;/or Micronutrient product</th>
<th>Placebo/Control</th>
<th>Mode of delivery</th>
<th>Total daily dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaidya et al., 2008</td>
<td>Yes</td>
<td>Prenatally only</td>
<td>Between 12-20 completed wks.</td>
<td>&lt;20 wks. to delivery</td>
<td>5-7</td>
<td>Vitamins A, E, D, B1, B2, niacin, B6, B12, folic acid, C + iron, zinc, copper, selenium, iodine</td>
<td>Current nationally advised tablets (iron 60 mg &amp; folic acid 400 μg)</td>
<td>Tablet</td>
<td>Recommended daily allowance of 15 vitamins &amp; minerals</td>
</tr>
<tr>
<td>Hawkesworth et al., 2008</td>
<td>Yes (Cluster randomization)</td>
<td>Prenatally only</td>
<td>20 wks</td>
<td>20 wks. to delivery</td>
<td>4.5-5</td>
<td>Biscuits containing roasted ground-nuts, rice flour, sugar, and groundnut oil</td>
<td>No treatment, supplement provided for 20wks after delivery</td>
<td>N/A</td>
<td>Two biscuits: of 4,250 kJ energy, 22g protein, 56g fat, 47mg calcium &amp; 1.8mg iron</td>
</tr>
<tr>
<td>Stewart et al., 2009</td>
<td>Yes Except for small differences in ethnic composition &amp; land holding + women in the control group weighed slightly less</td>
<td>Prenatally only</td>
<td>11.2-11.6</td>
<td>11wks. to delivery</td>
<td>6.5-7</td>
<td>Folic acid or folic acid + iron or folic acid + iron + zinc or multiple micronutrients containing folic acid, iron, zinc, 11 vitamins &amp; minerals***</td>
<td>Vitamin A alone (1000 μg)</td>
<td>Caplet</td>
<td>Folic acid 400μg, iron 60mg, zinc 30mg, 10mg Vit. D, 10mg Vit. E, 1.6mg thiamine, 1.8mg riboflavin, 20mg niacin, 2.2mg Vit. B6, 2.6μg Vit. B-12, 100mg Vit. C, 65 μg Vit. K, 2.0 mg Cu, 100mg Mg</td>
</tr>
<tr>
<td>Roth et al., 2013</td>
<td>Yes Mothers in</td>
<td>Prenatally only</td>
<td>From 26 &amp; &lt;30 wks.</td>
<td>Between 26-&lt;30 wks. to</td>
<td>2.5-3</td>
<td>Vitamin D3 (cholecalciferol) a Miglyol oil 812</td>
<td>Oil (the vehicle)</td>
<td>35,000 IU/wk</td>
<td></td>
</tr>
<tr>
<td>Vitamin D group had higher education level</td>
<td>delivery</td>
<td>high-concentration (20,000 IU D3 per mL) liquid formulation</td>
<td>used in Vigantol Oil</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>------------------------------------------</td>
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<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

*Indicates total duration in mother, infant or both, whichever is applicable
**Indicates total duration in pregnancy plus after birth, if applicable
***All supplements were given with 1000 μg retinol equivalent
Chapter 5: Assessing quality of maternal diet during pregnancy and infant feeding behaviours and their associations with infant birth weight Z-score and weight/age at 6 months of age

5.1. Overview of the chapter

This chapter describes a cohort study examining the quality of the diet in women during pregnancy as well as infant feeding behaviour and their associations with infants’ birth weight Z-scores and weight/age at 2-3 and 6 months of age. Women completed a validated Food Frequency Questionnaire in the last trimester of pregnancy (FFQ-P) and data on infant feeding behaviour was collected using a Milk Diary (MD) between 2-3 months of infant age. Data on infant weight were collected at 2-3 and 6 months follow-ups. The questionnaires used will be described and their choice justified. The results are presented and discussed in relation to the role of the quality of maternal diet and infant feeding behaviours and any implications the findings may have.

5.2. Objectives

a. To determine dietary quality of pregnant women during pregnancy and its association with birth weight Z-score
b. To determine the duration, number of feeds and volume of breast-feeding in infants and their associations with the weight/age of the infant at 2-3 and 6 months
c. To determine the timing of introduction of formula feeding, the volume consumed and number of feeds and their associations with the weight/age of the infant at 2-3 and 6 months
d. To determine weight/age differences of breast-fed, formula-fed and partially breast-fed infants at 2-3 and 6 months
e. To determine predictors of weight/age at 2-3 and 6 months age (infant feeding behaviour, maternal diet during pregnancy, age of introduction of solids).
5.3. Rationale for the choice of questionnaires

Questionnaires were selected if they had been validated in the target group and they were relevant for the study age group. In addition, the time needed to complete the questionnaires was considered. The following section provides detailed description of these questionnaires and the rationale for their choice.

5.3.1. Measuring maternal dietary intake

Measuring dietary intake, synonymous with dietary diversity or dietary variety, is defined by a simple count of foods or food groups consumed over a given reference period (Ruel, 2003). The assumption is that dietary variety and dietary quality are closely linked (Ruel, 2003); therefore measuring dietary variety offers an alternative for the nutritional quality and balance of food groups in the diet.

A number of methods have been developed for assessment of dietary intakes such as 24-hour dietary recall, food diary and Food Frequency Questionnaires (FFQs). It should be stressed that all methods have some limitations since eating is a complex behaviour influenced by a number of factors. When choosing a dietary assessment tool it is important to match the tool to the research question. For example food records, as a gold standard, provide detailed information on nutritional intake; however when data on usual intake or dietary patterns is required a FFQ could provide more accurate information over a period of time.

In this PhD study, a FFQ was used since the objective of the study was to measure overall dietary intake of women during pregnancy as opposed to quantifying nutritional intake. FFQs could be administered to provide data on overall habitual diet over time, minimising the degree of dietary recall required. There are a number of FFQs specifically designed to measure defined outcomes in pregnant women, adults and children. Cohort studies commonly use variations of the FFQ to assess dietary intake in populations (Siri-tarino, Sun, Hu, & Krauss, 2010). A systematic review on the validity and reliability of self-reported measures of foods and nutrients in pregnancy showed that FFQs had adequate validity in comparison to biomarkers, 24-hour recall and food records (Vézina-Im & Robitaille, 2014). The results of this review show that FFQs are validated and reliable measures for assessing dietary patterns in pregnant women. The questionnaire administered in this PhD project
(appendix 5.1) was an amended version of the Southampton Women’s Survey FFQ in pregnancy (Robinson, Godfrey, Osmond, Cox, & Barker, 1996). The main reason for selecting this particular questionnaire was because it was developed using the food lists from the UK. It has been validated in a group of pregnant women against a four-day weighed food diary in a geographical population similar to this study population. The original questionnaire was semi-quantitative (asking respondents to report food portion sizes in addition to frequency intake) and for this study, the portion sizes were removed since nutritional intake was not being assessed. Therefore, the questionnaire only asked for the frequency of intake and consisted of a list of 100 foods and drinks, divided into subcategories. The food list was also adapted, by input from a dietician, to reassure acceptable accuracy of dietary assessment. This was done by removing some unpopular/uncommon items e.g. haggis and adding some more common foods and drinks e.g. pizza/quiches/cheese flans, green tea. The subcategories of food and drinks were: starch and carbohydrate (13 foods), vegetables (17 items), fruits (11 items), drinks (10 items), meat and substitute (15 foods), fish (four items), egg and dairy (nine items), sweet and miscellaneous foods (17 items) and fat consumed separately as spreading, frying and other oil (three open questions to specify the type). Also, there was an additional free text food category to add any other foods that were eaten at least 1-2 times per week. The frequency of consumption over the last three months of each food and drink were recorded using a multiple response grid. The frequency options were: never, once/2-3 per months, once/month, once/fortnight, 1-2 times/week, 3-6 times/week, once/day and more than once a day. The intake options for the consumption of milk were: <75ml, 75-149ml, 150-300ml and >400ml. The respondents were also asked about the type of milk that was predominantly drunk. Women were asked to indicate the frequency of each item by ticking the appropriate box.

5.3.2. Measuring infant feeding behaviours

The type and amount of feed the infant receives is the primary driver of early infant growth. There are no gold standards for choosing a dietary assessment method in infants and all methods have some inherent flaws.

The questionnaire chosen for the second study of this PhD project was developed by Grimshaw (Grimshaw, 2012) as a valid and reliable instrument for collecting the
required weekly feeding practices of an infant. An adapted version of the questionnaire (4-days a week as opposed to 7-days) was developed for this study (appendix 5.2). This was mainly for convenience of the mothers as it has been shown that a 4-day timeframe is as informative as a longer 7-day diary (Livingstone & Robson, 2000). Mothers were asked to record the infant diet at four time points (morning 5am-12pm, afternoon 12-4pm, evening 4-10pm, night 10pm-5am) for each of the four days. Women were asked to record the volume and number of formula feeds and if breast-fed, the duration and times of breast-feeding at each time point. Questions were also included about age of introduction of formula, any drinks and solids. Parents were additionally asked to report infant weight as recorded in their red book, the infant personal child health record.

5.4. Methods

5.4.1. Study design and setting
This was a cohort study of pregnant women and their children followed-up until 6 months of age and was conducted as part of the Portsmouth Birth Cohort (PBC) registry. The settings for recruitment of pregnant women and follow-ups of their children were the antenatal NHS clinic in Queen Alexandra (QA) hospital, Portsmouth and within the community, respectively.

5.4.2. Study sample and recruitment
The target population for the PBC database registry were pregnant women attending antenatal NHS clinics in QA hospital, Portsmouth, at any stage in pregnancy. For this second study, a convenient sample of pregnant women that were due to give birth between February-August 2016 and their newborns were included. The study flowchart is presented in Figure 5.1. The inclusion criteria were defined as: being over 16 years of age, resident in Portsmouth city area only, being able to understand, write and speak English. Exclusion criteria was pregnancies that were terminated due to late abortion or stillbirth and an extra exclusion criterion for this PhD project was defined for infants with a suspicion of congenital abnormality or diagnosed with congenital abnormality/chronic condition since these are likely to influence normal development (including feeding behaviour).
Women were approached by the study midwives at QA hospital at the time of their routine visit to the antenatal NHS clinic and were provided with the study information sheet and consent form (appendices 5.3 and 5.4). Women were either consented on the same day or were able to return the consent form via post at a later time. Enrolled women consented to be contacted for further studies.
5.4.3. Ethical considerations

Ethical approval for the PBC registry was granted from Berkshire NHS Research Ethics Committee on 23rd January 2015 (appendix 5.5). The registry included two waves, known as wave 0 and wave 1, which collected data during pregnancy and birth.
respectively using the recruitment and birth questionnaires (appendices 5.6 and 5.7). In order to ask participants to complete the additional questionnaires for this study (FFQ-P at second and third trimesters of pregnancy and MD at 1 and 4 months age) a substantial amendment form was submitted to the Berkshire NHS ethics committee. Further ethical approval for the revised birth cohort registry questionnaires was obtained on 12th May 2015 (appendix 5.8). The NHS R&D approval from the QA hospital, as the setting for conducting the PBC birth cohort, was obtained on 11th December 2015, following the initial communications with the QA hospital in February 2015 (appendix 5.9). Following both the NHS R&D and ethical approval, recruitment of pregnant women began in December 2015.

Two further amendments were requested from the NHS ethics committee and were granted on 28th January 2016 and 9th May 2016 (appendices 5.10 and 5.11). Firstly, it was requested that the FFQ-P be completed only at the third trimester of pregnancy, since the two initial planned time points were quite close and also, data on the second trimester could not really reflect the real dietary pattern of pregnant women due to morning sickness. Secondly, a change was made to the length of the MDs questionnaire and the time they were collected, amended to when the infants were 2-3 months of age. This change was because a few mothers that were contacted when their baby was 1-month old did not respond and one withdrew from the study. It was hypothesised that the questionnaire was too long and that women found it difficult to participate in the study during this hectic transition in their life. An additional ethical approval was also requested for the 6-months follow-up questionnaire and obtained on 8th July 2016 (appendix 12).

Data for the PBC registry was created using Microsoft Access software version 10 and was password protected. Data was anonymised and when not in use, secured in locked filing cabinets. Online questionnaires were administered via Bristol Online Surveys (BOS) (http://www.survey.bris.ac.uk), for which there is encryption, ensuring the data cannot be intercepted by third parties. Women were free to withdraw from the study at any time.
5.4.4. Administration of questionnaires

At the time of enrolment into the PBC database registry, midwives completed the wave 0 questionnaire (appendix 5.6) that included several sections collecting information such as demographic data, history of mental and physical health problems. Maternal and paternal occupations were captured using an open question and grouped based on the coding of the Office for National Statistics’ (ONS) Standard Occupational Classification (SOC) hierarchy. The SOC hierarchy has 9 major occupational groups and replies that could not be coded using the SOC coding system were grouped separately as “not classified”. Other codes defined were: unemployed, student, housewife and missing replies. The wave 1 questionnaire collected data on pregnancy and birth outcomes (appendix 5.7), which was filled in by the midwives consulting the medical records at QA hospital.

The questionnaires for this PhD project, namely the FFQ-P and MD were both self-administered and women were provided with contact details of the research team to clarify any queries. Women received the FFQ-P in their third trimester of pregnancy and based on their preference, it was completed either online or as a postal questionnaire. Online reminders were sent to non-respondents within 5-7 days of the date of first email. If no replies within a week, they received a phone call to ask if they were willing to take part and whether they wished to receive the questionnaire online or by post. Women who received the FFQ-P via post were given three weeks to return the completed questionnaire. The non-respondents received a reminder phone call and if necessary, a second questionnaire was posted. The questionnaires were posted out along with prepaid addressed return envelopes.

MDs and a prepaid envelope were posted to mothers when their babies were two months of age, and if they had not responded within three weeks they received a phone reminder.

5.4.5. Questionnaire coding

Where possible, questionnaires were coded and scored according to published standards. To ensure consistency in coding of questionnaires, a coding logbook was maintained. The data from a questionnaire were used if it was at least 75% complete.
5.4.5.1. Food Frequency Questionnaire in Pregnancy (FFQ-P)

The food items in the administered FFQ-P were adjusted with the Alternative Healthy Eating Index (AHEI) (Rifas-Shiman, Rich-Edwards, Kleinman, Oken, & Gillman, 2009). The AHEI measures diet quality on a 90-point scale with nine components, each contributing 10 possible points: vegetable, fruits, ratio of white to red meat, fibre, trans fat, ratio of PUFA to saturated fatty acids; folate, calcium and iron from foods. The AHEI components were adjusted to our FFQ-P since our questionnaire only measured the frequency of food items and not portion size; therefore it was not possible to calculate the nutrient intake and also ratio for the components from foods.

In total, given that the FFQ-P administered in this study is a quantitative measure, including 99 food items and also taking into account the UK guidelines for a healthy diet in pregnancy, we defined seven AHEI components from the FFQ-P as follows: vegetable and fruit, iron-rich foods, fibre, dairy, fish, fat and Vitamin D. As per the UK guidelines, recommended healthy eating during pregnancy is: at least five portions a day of fruit and vegetables, three servings a day of iron-rich foods, daily consumption of fibre from sources rich in fibre such as vegetable/fruit and starchy foods, four servings a day of dairy products, two portions of fish a week including one oily fish, low consumption of fatty foods and fats particularly from saturated sources and daily intake of 10μgram of Vitamin D. Consumption of each food item was calculated per week since the recommended criteria for intake of different food groups varies such as daily intake of fruit and vegetables versus weekly intake of fish.

To reflect consumption per week, a numeric value was assigned to each frequency category, ranging from 0 to 14 (never=0, once every 2-3 months=0.1, once a month=0.25, once a fortnight=0.5, 1-2 times per week=1.5, 3-6 times per week=4.5, once a day=7 and more than once a day=14). The frequencies of each contributing food item in the components were added to calculate the weekly frequency intake of food components (vegetable/fruit, iron-rich foods, fibre, dairy). The iron-rich food component included red meat items defined as pork, lamb, beef and minced meat dishes as well as beans/pulses and spinach. Other meats (gammon/bacon, ham, sausages and meat pies) were defined as processed meat and were not considered in this component.

Missing replies to food items were replaced in the following way: if there were ≥25% of missing data, the missing replies were not calculated. For missing replies <25%,
scores of the food items included in each component were summed and divided by the total replies and the mean frequency was replaced for the missing reply. One participant did not reply to any vegetable items and for this woman we calculated the number of intakes of fruits only per week. The number of intakes of fruit that she had per week (55.5) equals 7.9 per day. This was higher than the number of portions of fruit/vegetables to score the maximum score of 10 for the healthy eating index. So she was included in the analysis with the maximum score of 10. One participant also did not reply to two out of five questions for the fibre component, which was greater than 25% and therefore, the missing scores were not calculated for this woman.

The total score of each component was from 0 to 10 where a score of 10 indicates that the participant meets the UK guidelines for healthy eating. The intermediate scores were calculated proportionately based on the recommended daily serving of food items as per the UK guideline, converted for weekly consumption. For example, for a participant with \( \geq 35 \) frequency consumption of vegetable/fruit per week (five portions a day), a total score of 10 was assigned. Similarly, vegetable/fruit frequency consumption of 0, 7 and \(<14\); 14 and \(<21\); 21 and \(<28\); and 28 and \(<35\) per week were assigned a score of 0, 2, 4, 6 and 8, respectively. For scoring the fibre component, we looked at the distribution of raw scores in our sample since there is no specific guideline for daily consumption of fibre. The frequency consumption of fibre per week in our sample were 0 to \(<7\), 7 to \(<14\), 14 to \(<21\), 21 to \(<28\), 28 to \(<35\) and 35 (five servings a day) which were assigned a total score of 0, 2, 4, 6, 8 and 10 respectively. A logbook was kept to assure the consistency of scoring for all components.

Calculation of total scoring for fish, fat and Vitamin D components were conducted differently since this involved extrapolating the scores from the responses given to open questions. These are described below:

- Fish component: intake of fish was considered as the main source of omega-3 fatty acids. The intake of white and oily fish, one intake each per week as per the UK guideline, was considered for scoring. At the same time, if women had specified that they took pregna-care and/or pregna-max that includes omega-3, they were also given a score of 10. The general agreement for the scoring was as follows: 2 intakes of oily fish got a score of 10. Taking only an omega-3
vitamin source got a score of 10. One intake of oily fish and one intake of white fish got a score of 10. One intake of oily fish only got a score of 5 and one or more intake of white fish with no portion of oily fish got a score of 5. Less than one intake of fish got a score of 0. Participants consuming omega-3 as well as oily fish once or more per week were also assigned a score of 10.

- **Fat component:** participants were asked to specify the type and frequency of spreading, frying fat/oil and other oil separately. There were a lot of missing replies to the “other oil” question and the specified replies were mayonnaise and different salad dressings; therefore replies to this question were not included in the calculation. The replies to “spreading and frying fat” greatly varied and included two missing replies to one or both questions. Using an Internet search, we identified the percentage of saturated fat for each brand. The agreement for scoring was as follows: for each spreading and frying fat/oils, saturated fat of greater than 50% got a score of 0, between 25-50% got a score of 5 and less than 25% got a score of 10. Combined replies of lower and higher saturated fat sources such as butter or margarine got a score of 5. For general replies for some brands such as fry light oil, they got an average score of 5 because there are 3 different varieties of the brand. Women that replied “never used” the spreading/frying oil were given a score of 10. Missing replies to either or both fat questions were considered as a score of 0 assuming that those had unhealthy habits. We did not consider frequency of the fats consumed per week for scoring because we did not have information for portion size. Therefore, the scoring was only conducted for the type of fat and total score was calculated by adding the scores for spreading and frying oil together and divided by two.

- **Vitamin D:** this component was considered for the intake of Vitamin D and participants were asked whether they took any nutritional supplements including vitamins and if yes, to specify the type. The replies were diverse and there were 19 missing replies. The agreements for scoring were as follows: participants who specified taking multivitamins/Vitamin D were given a score of 10. Participants with either a missing reply or those who did not specify the type were scored 0. It was also assumed that women consumed the specified multivitamin(s) daily. Three women replied “yes”, they took supplements but
did not specify the type and got a score of 0. We were not able to calculate intermediate scores for this component and the total scoring was either 0 or 10.

The scores of all components were added together to obtain the total diet quality score that ranged from 0 (lowest) to 70 (highest), indicating women with higher scores had a better quality diet and met the recommendations partially to fully. There was one missing score relating to one participant with two missing replies for the fibre component. This was replaced by the mean of her other six calculated components. The food items included in each food component and number of missing replies are presented in Table 5.1.

### Table 5.1. Food items included in each food component

<table>
<thead>
<tr>
<th>Food group</th>
<th>Food items included</th>
<th>Missing replies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegetable/fruit</td>
<td>Questions (Qs) 14 to 41 (28 items)</td>
<td>5</td>
</tr>
<tr>
<td>Rich-iron foods</td>
<td>Qs 18, 20, 53, 58-60 (6 items)</td>
<td>0</td>
</tr>
<tr>
<td>Fibre</td>
<td>Qs 2-5 &amp; 12 (5 items)</td>
<td>2</td>
</tr>
<tr>
<td>Dairy</td>
<td>Qs 73-78 (6 items)</td>
<td>0</td>
</tr>
<tr>
<td>Fish</td>
<td>Qs 67-69 (3 items)</td>
<td>0</td>
</tr>
<tr>
<td>Fat</td>
<td>Qs 97 &amp; 99 (3 items)</td>
<td>2</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>Q 5.4 (1 item)</td>
<td>19</td>
</tr>
</tbody>
</table>

The “unhealthy eating behaviours” were also considered from the other items included in the FFQ-P. These were defined as sugar consumption (including 15 items of cola, soft and energy drinks, drinking chocolate and sweets), processed food (including 4 items of sausages, ham, bacon/gammon and meat pies) and junk foods (including 4 items of take away/fast food, ready meals, crisps/savoury snacks and roast potatoes/chips). The frequency of consumption of these items were calculated per week using the same method described for AHEI-P components; however we did not calculate a total score from 0 to 10 for these components and they were treated as continuous variables. Therefore, higher frequency intake of unhealthy items equates to a higher score.

### 5.4.5.2. Milk Diary (MD)

The raw data for the milk diary was coded and the mean values were calculated allowing the resultant variables to be analysed in a number of different ways. Method of feeding was categorised as exclusive breast-feeding, formula feeding and partially breast-fed (for any infant that has ever been introduced to formula). Mean duration
and number of breast-feeds were calculated. The volume of breast milk intake per day for each infant was calculated for the infant’s age according to the standard values for the daily mean intake (Haisma et al., 2003; Paul, Black, Evan, Cole, & Whitehead, 1988). The quoted average breast milk intake by age is as follow:

- **0-2 months**: 2-5oz per feeding, 26oz per day (736 gram)
- **2-4 months**: 4-6oz per feeding, 30oz per day (850 gram)
- **4-6 months**: 5-7oz per feeding, 31oz per day (878 gram)

The volume of formula milk consumed was converted to millilitres and mean values for the volume and number of formula feeds per day were calculated. For partially breast-fed infants, the standard daily volume of breast milk for age was subtracted from the formula taken. If the baby had consumed water, this was not included in the total mean calculated for either formula or breast milk intake.

Infant weight was recorded at 2-3 and 6 months follow-ups using the information from the baby’s red book. Data on introduction of solids into an infant diet and the age of introduction were collected at the 6-months follow-up questionnaire.

### 5.4.6. Data analysis

The Statistical Package for Social Sciences (SPSS) software (IBM, version 23) was used to analyse the data. Data sets were checked for outliers. Weight measurements at birth and the two follow-up time points were converted into weight/age using the LMS Growth software (Cole, 1990). Descriptive statistics were used to describe the data and all continuous variables were tested for normality using histograms. The mean and SD were presented for normally distributed data and otherwise, median and range reported. Categorical variables were expressed as frequency and percentage. Spearman bivariate correlations were used to assess the relations between the food components, since some were not normally distributed. The $\chi^2$ test was used for categorical variables. One-way ANOVA analysis was performed to measure the effect of one categorical variable such as attention to diet and infant feeding behaviour with the continuous outcome(s). To assess the effect of multiple factors on the main outcome measures, multiple regression analyses were conducted. The significance level for all analyses was defined at the 0.05 level.
Multiple demographic and behavioural factors during pregnancy such as smoking, maternal age, and education could contribute towards the quality of maternal diet and thus, the risk of developing childhood obesity. Investigating the detailed associations of these factors on infant weight was not within the remit of the current study. There were also a few smokers (5%) among mothers who responded to the milk diary questionnaire and additionally, no correlations were found between maternal age and education with AHEI-P score and unhealthy dietary habits. Therefore, the regression models were not adjusted for these factors.

5.5. Results

Pregnant women with the expected date of delivery between mid February to 30\textsuperscript{th} August 2016 were approached to take part in this project. A total of 134 pregnant women were consented to take part in this project. Women who were recruited at very late stages of pregnancy (32-39 gestation weeks) were not invited to complete the FFQ-P and therefore, a total of 103 women were asked to complete the FFQ-P. Four women were not sent the MDs due to delays in obtaining NHS Health Research Authority (HRA) approval and also, one baby was born prematurely and was still in hospital at 2 months of age. Hence, a total of 129 pregnant women were invited to respond to the MD.

Figure 5.2. Replies to study questionnaires
5.5.1. Description of study sample

Detailed demographic characteristics of the whole sample and those who responded to each questionnaire are shown in Table 5.2. Where there were missing replies to any of the reported outcomes, these are specified. Continuous data (maternal age and number of children) were checked for normality and where not normally distributed, median reported.

Maternal age was normally distributed, whereas data for number of children was skewed to the left, indicating that most participants had smaller families. Of the sample invited, 61.2% participants were married and 31.3% women and 22.4% of their husbands/partners reported having professional jobs. Also, 53% of women were educated with university degrees while 39.6% of the fathers had university degrees. 82.1% were white British and 58.2% reported having no religion.

There were statistically significant differences between responders and non-responders as more of the responders had higher professional roles and educational level, were married and also owned a property.

Table 5.2. Demographic characteristics of the respondents

<table>
<thead>
<tr>
<th></th>
<th>Whole sample (N=134)</th>
<th>FFQ-P (N=94)</th>
<th>Milk Diary (N=79)</th>
<th>6-month F-U (N=80)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) [Mean (SD)]</td>
<td>30.30 (4.98)</td>
<td>31.09 (4.25)</td>
<td>31.37 (4.22)</td>
<td>31.22 (4.48)</td>
</tr>
<tr>
<td>Mother’s job (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Managers, directors &amp; senior officials</td>
<td>9 (6.7)</td>
<td>5 (5.3)</td>
<td>5 (6.3)</td>
<td>7 (8.7)</td>
</tr>
<tr>
<td>Professional occupations</td>
<td>42 (31.3)</td>
<td>37 (39.4)</td>
<td>34 (43.0)</td>
<td>34 (42.5)</td>
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<tr>
<td>Associate professional &amp; technical occupations</td>
<td>12 (9.0)</td>
<td>9 (9.6)</td>
<td>5 (6.3)</td>
<td>7 (8.7)</td>
</tr>
<tr>
<td>Administrative &amp; secretarial occupations</td>
<td>14 (10.4)</td>
<td>12 (12.8)</td>
<td>10 (12.7)</td>
<td>8 (10.0)</td>
</tr>
<tr>
<td>Skilled trade occupations</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caring, leisure &amp; other service occupations</td>
<td>10 (7.5)</td>
<td>5 (5.3)</td>
<td>5 (6.3)</td>
<td>4 (5.0)</td>
</tr>
<tr>
<td>Sales &amp; customer service occupations</td>
<td>6 (4.5)</td>
<td>4 (4.3)</td>
<td>3 (3.8)</td>
<td>3 (3.7)</td>
</tr>
<tr>
<td>Process, plant &amp; machine operatives</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elementary occupations</td>
<td>6 (4.5)</td>
<td>4 (4.3)</td>
<td>3 (3.8)</td>
<td>2 (2.5)</td>
</tr>
<tr>
<td>Unemployed</td>
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<td>3 (3.2)</td>
<td>3 (3.8)</td>
<td>3 (3.8)</td>
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<tr>
<td>Student</td>
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<td>1 (1.1)</td>
<td>1 (1.3)</td>
<td>1 (1.3)</td>
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<tr>
<td>Housewife</td>
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</tr>
<tr>
<td></td>
<td>Whole sample (N=134)</td>
<td>FFQ-P (N=94)</td>
<td>Milk Diary (N=79)</td>
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<td>Father’s job (%)</td>
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<tr>
<td>Managers, directors &amp; senior officials</td>
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<td>9 (9.6)</td>
<td>6 (7.6)</td>
<td>9 (11.3)</td>
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<td>14 (14.9)</td>
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<tr>
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<td>2 (2.1)</td>
<td>2 (2.5)</td>
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<tr>
<td>Sales &amp; customer service occupations</td>
<td>4 (3.0)</td>
<td>2 (2.1)</td>
<td>----</td>
<td>1 (1.3)</td>
</tr>
<tr>
<td>Process, plant &amp; machine operatives</td>
<td>6 (4.5)</td>
<td>2 (2.1)</td>
<td>3 (3.8)</td>
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<td>2 (2.1)</td>
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<td>2 (2.5)</td>
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<tr>
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<td>5 (3.7)</td>
<td>3 (3.2)</td>
<td>2 (2.5)</td>
<td>---</td>
</tr>
<tr>
<td>Student</td>
<td>2 (1.5)</td>
<td>1 (1.1)</td>
<td>----</td>
<td>---</td>
</tr>
<tr>
<td>Not classified</td>
<td>13 (9.7)</td>
<td>7 (7.4)</td>
<td>6 (7.6)</td>
<td>7 (8.7)</td>
</tr>
<tr>
<td>Missing</td>
<td>7 (5.2)</td>
<td>1 (1.1)</td>
<td>----</td>
<td>1 (1.3)</td>
</tr>
<tr>
<td>Mother’s Education (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>School</td>
<td>9 (6.7)</td>
<td>3 (3.2)</td>
<td>2 (2.5)</td>
<td>2 (2.4)</td>
</tr>
<tr>
<td>Further (After GCSEs)</td>
<td>52 (38.8)</td>
<td>30 (31.9)</td>
<td>23 (29.1)</td>
<td>24 (30.0)</td>
</tr>
<tr>
<td>Higher (University)</td>
<td>71 (53.0)</td>
<td>59 (62.8)</td>
<td>53 (67.1)</td>
<td>53 (66.3)</td>
</tr>
<tr>
<td>Don’t know</td>
<td>----</td>
<td>----</td>
<td>1 (1.3)</td>
<td>1 (1.3)</td>
</tr>
<tr>
<td>Missing</td>
<td>2 (1.5)</td>
<td>2 (2.1)</td>
<td>----</td>
<td>---</td>
</tr>
<tr>
<td>Father’s education (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>School</td>
<td>20 (14.9)</td>
<td>12 (12.8)</td>
<td>10 (12.7)</td>
<td>6 (7.5)</td>
</tr>
<tr>
<td>Further (After GCSEs)</td>
<td>54 (40.5)</td>
<td>31 (33.0)</td>
<td>31 (39.2)</td>
<td>32 (40.0)</td>
</tr>
<tr>
<td>Higher (University)</td>
<td>53 (39.6)</td>
<td>48 (51.1)</td>
<td>37 (46.8)</td>
<td>40 (50.0)</td>
</tr>
<tr>
<td>Don’t know</td>
<td>2 (1.5)</td>
<td>1 (1.1)</td>
<td>1 (1.3)</td>
<td>2 (2.5)</td>
</tr>
<tr>
<td>Missing</td>
<td>5 (3.7)</td>
<td>2 (2.1)</td>
<td>----</td>
<td>---</td>
</tr>
<tr>
<td>Marital status (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Domestic partnership</td>
<td>42 (31.3)</td>
<td>28 (29.8)</td>
<td>20 (25.3)</td>
<td>26 (32.4)</td>
</tr>
<tr>
<td>Married</td>
<td>82 (61.2)</td>
<td>62 (66.0)</td>
<td>57 (72.2)</td>
<td>53 (66.3)</td>
</tr>
<tr>
<td>Single</td>
<td>10 (7.5)</td>
<td>4 (4.3)</td>
<td>2 (2.5)</td>
<td>1 (1.3)</td>
</tr>
<tr>
<td>No. of children (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Median (range)]</td>
<td>1.0 (0-4)</td>
<td>1.0 (0-3)</td>
<td>1.0 (0-3)</td>
<td>1.0 (0-3)</td>
</tr>
<tr>
<td>Type of property (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Own home</td>
<td>77 (57.5)</td>
<td>61 (64.9)</td>
<td>55 (69.6)</td>
<td>55 (68.7)</td>
</tr>
<tr>
<td>Parents home</td>
<td>5 (3.7)</td>
<td>3 (3.2)</td>
<td>2 (2.5)</td>
<td>1 (1.3)</td>
</tr>
<tr>
<td>Housing association</td>
<td>10 (7.5)</td>
<td>6 (6.4)</td>
<td>2 (2.5)</td>
<td>3 (3.8)</td>
</tr>
<tr>
<td>Private rent</td>
<td>32 (23.9)</td>
<td>18 (19.1)</td>
<td>14 (17.7)</td>
<td>14 (17.5)</td>
</tr>
<tr>
<td>Other</td>
<td>10 (7.7)</td>
<td>6 (6.4)</td>
<td>6 (7.6)</td>
<td>7 (8.7)</td>
</tr>
<tr>
<td>Ethnicity (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White (English/Welsh/Scottish/Northern Irish)</td>
<td>110 (82.1)</td>
<td>79 (84.0)</td>
<td>63 (79.7)</td>
<td>63 (78.6)</td>
</tr>
<tr>
<td>White (Irish/any other)</td>
<td>15 (11.1)</td>
<td>12 (12.7)</td>
<td>13 (16.4)</td>
<td>14 (17.6)</td>
</tr>
<tr>
<td>Other ethnic groups</td>
<td>8 (6.10)</td>
<td>2 (2.2)</td>
<td>2 (2.6)</td>
<td>2 (2.5)</td>
</tr>
<tr>
<td>Missing</td>
<td>1 (0.7)</td>
<td>1 (1.1)</td>
<td>1 (1.3)</td>
<td>1 (1.3)</td>
</tr>
<tr>
<td>Religion (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Christian</td>
<td>40 (29.8)</td>
<td>29 (30.9)</td>
<td>24 (30.4)</td>
<td>26 (32.5)</td>
</tr>
<tr>
<td>No religion</td>
<td>78 (58.2)</td>
<td>53 (56.4)</td>
<td>45 (57.0)</td>
<td>43 (53.6)</td>
</tr>
<tr>
<td>Hindu</td>
<td>2 (1.5)</td>
<td>1 (1.1)</td>
<td>1 (1.3)</td>
<td>1 (1.3)</td>
</tr>
<tr>
<td>Muslim</td>
<td>1 (0.7)</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Other</td>
<td>1 (0.7)</td>
<td>1 (1.1)</td>
<td>1 (1.3)</td>
<td>1 (1.3)</td>
</tr>
<tr>
<td>Not stated</td>
<td>12 (9.0)</td>
<td>10 (10.5)</td>
<td>8 (10.1)</td>
<td>9 (11.3)</td>
</tr>
</tbody>
</table>
Details of pregnancy and birth outcomes are presented in Table 5.3. One mother withdrew before giving birth and therefore, data on pregnancy and birth outcomes were not available. There were two sets of twins and hence, data for pregnancy and birth outcomes were available for 133 mothers and 135 babies, respectively. Where there were missing replies to any of the reported outcomes, these are specified. Measurement of birth weight (kg) was within the normal range and mean and SD are reported. The majority of babies were born at term and were normally delivered. They were all live births and mean birth weight was 3.32kg.
Table 5.3. Pregnancy and birth related outcomes

<table>
<thead>
<tr>
<th>Pregnancy-related outcomes</th>
<th>All sample N=133* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age at birth (weeks) (n=127)</td>
<td>112 (83.6)</td>
</tr>
<tr>
<td>Term &amp; post-term babies</td>
<td></td>
</tr>
<tr>
<td>Late preterm (34-37 weeks)</td>
<td>14 (10.4)</td>
</tr>
<tr>
<td>Extreme premature (&lt;28 weeks)</td>
<td>1 (0.7)</td>
</tr>
<tr>
<td>Missing</td>
<td>7 (5.2)</td>
</tr>
<tr>
<td>Single birth</td>
<td>131 (98.5)</td>
</tr>
<tr>
<td>Type of delivery</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>75 (56.4)</td>
</tr>
<tr>
<td>Caesarean</td>
<td>45 (33.8)</td>
</tr>
<tr>
<td>Instrumental</td>
<td>13 (9.8)</td>
</tr>
<tr>
<td>Perinatal trauma or episiotomy (n=130)</td>
<td></td>
</tr>
<tr>
<td>1\textsuperscript{st} degree</td>
<td>15 (11.3)</td>
</tr>
<tr>
<td>2\textsuperscript{nd} degree</td>
<td>34 (25.6)</td>
</tr>
<tr>
<td>3\textsuperscript{rd} degree</td>
<td>1 (0.8)</td>
</tr>
<tr>
<td>Episiotomy</td>
<td>15 (11.3)</td>
</tr>
<tr>
<td>None</td>
<td>65 (48.9)</td>
</tr>
<tr>
<td>Missing</td>
<td>3 (2.3)</td>
</tr>
<tr>
<td>No. of days mother spent at QA hospital (N=91) [Mean (range)]</td>
<td>2.15 (1-9)</td>
</tr>
<tr>
<td>Birth-related outcomes</td>
<td>N=135***</td>
</tr>
<tr>
<td>Birth weight [kg] [Mean (SD)]</td>
<td>3.32 (0.60)</td>
</tr>
<tr>
<td>Range</td>
<td>(0.650-4.52)</td>
</tr>
<tr>
<td>Birth weight Z-score (SD units) (n=134)</td>
<td>-0.087 (0.95)</td>
</tr>
<tr>
<td>Birth weight Z-score (SD units), term babies (n=112)</td>
<td>0.038 (0.88)</td>
</tr>
<tr>
<td>Gender (n=134)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>68 (50.4)</td>
</tr>
<tr>
<td>Female</td>
<td>66 (48.9)</td>
</tr>
<tr>
<td>Missing</td>
<td>1 (0.7) ***</td>
</tr>
<tr>
<td>Method of feeding on 1\textsuperscript{st} day of birth</td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>101 (74.8)</td>
</tr>
<tr>
<td>Bottle</td>
<td>27 (20.0)</td>
</tr>
<tr>
<td>Both</td>
<td>6 (4.4)</td>
</tr>
<tr>
<td>Missing</td>
<td>1 (0.7)</td>
</tr>
<tr>
<td>Apgar score</td>
<td></td>
</tr>
<tr>
<td>1 minute [mean (range)] (n=135)</td>
<td>8.62 (2-10)</td>
</tr>
<tr>
<td>5 minutes [mean (range)] (n=134)</td>
<td>9.17 (5-10)</td>
</tr>
<tr>
<td>Admitted to Neonatal Intense Care Unit</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>13 (9.6)</td>
</tr>
<tr>
<td>No</td>
<td>119 (88.2)</td>
</tr>
<tr>
<td>Missing</td>
<td>3 (2.2)</td>
</tr>
</tbody>
</table>

\*One mother withdrew before giving birth
\**Includes 2 sets of twins
\***One baby was born in Birmingham and the staff did not have access to hospital system

5.5.2. FFQ-P data and the Healthy Eating Index score

Of the 103 pregnant women that were asked to complete the FFQ-P, 94 responded (91.26%). Fifty-five (58.5%) of the respondents reported that they excluded foods from their diet and of these, over 90% specified the reason was based on the guidelines and recommendations during pregnancy. Other reasons were: being allergic (two), vegetarian (two) and coeliac disease (one). The main food groups excluded were raw/rare meat, soft cheese, eggs, shellfish and liver/pate. In response to
the question ‘How much attention do you pay to your diet in terms of healthy eating?’, 7 (7.6%) and 70 (76.1%) replied very little and somewhat, respectively. Only 15 women (16.3%) specified “a great deal” and two women did not respond. Also 62.1% of participants reported that they have taken any medications during pregnancy. Table 5.4 describes the list of medications taken during pregnancy. Missing replies are not reported in the table. Other medications were mainly reported as antiemetic and digestive tablets and nutritional supplements mostly multivitamins and iron/folic acid tablets.

Table 5.4. Medications taken during pregnancy

<table>
<thead>
<tr>
<th>Medications during pregnancy N (%)</th>
<th>Antibiotics (n=67)</th>
<th>Paracetamol (n=68)</th>
<th>Aspirin (n=60)</th>
<th>Other medications (n=63)</th>
<th>Nutritional supplements (n=75)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>21 (31.3)</td>
<td>47 (69.1)</td>
<td>3 (5.0)</td>
<td>25 (39.7)</td>
<td>58 (77.3)</td>
</tr>
<tr>
<td>No</td>
<td>46 (68.7)</td>
<td>21 (30.9)</td>
<td>57 (95.0)</td>
<td>38 (60.3)</td>
<td>17 (22.7)</td>
</tr>
</tbody>
</table>

Criteria for scoring the AHEI-P is described in section 5.4.5.1, where the minimum and maximum possible scores were 0-70 and a higher score indicates a higher level of healthy eating. Mean of the total AHEI-P score was 33.60. The frequency intake of food components and their total is shown in Table 5.5. Missing replies to food items were as follows: vegetable/fruit (5), fibre (2) and sugar (6) items.
Table 5.5. Mean frequency intakes in last trimester of pregnancy and AHEI-P scores of 94 pregnant women who responded

<table>
<thead>
<tr>
<th>Food component</th>
<th>Frequency intakea</th>
<th>Criterion for min. score of 0b</th>
<th>Criterion for max. score of 10</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td></td>
<td></td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>Vegetable/Fruit</td>
<td>48.42 (20.49)</td>
<td>&lt;7</td>
<td>≥35</td>
<td>8.87 (2.04)</td>
</tr>
<tr>
<td>Iron rich</td>
<td>10.17 (5.33)</td>
<td>0</td>
<td>3</td>
<td>3.26 (2.80)</td>
</tr>
<tr>
<td>Dairy</td>
<td>14.44 (6.50)</td>
<td>0</td>
<td>≥28</td>
<td>3.90 (2.52)</td>
</tr>
<tr>
<td>Fibre</td>
<td>9.80 (7.53)c</td>
<td>0</td>
<td>≥35</td>
<td>1.99 (2.04)</td>
</tr>
<tr>
<td>Fat</td>
<td>---</td>
<td>0</td>
<td>10</td>
<td>7.36 (2.89)</td>
</tr>
<tr>
<td>Fish (omega-3)d</td>
<td>---</td>
<td>0</td>
<td>10</td>
<td>3.29 (3.91)</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>---</td>
<td>0</td>
<td>10</td>
<td>4.89 (5.02)</td>
</tr>
<tr>
<td>Total AHEI-P score</td>
<td></td>
<td></td>
<td></td>
<td>33.60 (8.93)</td>
</tr>
</tbody>
</table>

- Frequencies were normally distributed and mean (SD) reported
- Intermediate frequency intakes were proportionately scored between 0 and 10
- Not possible to calculate the frequency and only scoring
- The distribution of fibre component was partially normal and the median (range)=9.24 (0-35)

Unhealthy eating habits were treated as continuous variables and scores represent number of times unhealthy foods consumed per week. The median frequency intake of unhealthy eating habits is presented in Table 5.6.

Table 5.6. Median frequency intakes of unhealthy eating habits in last trimester of pregnancy in participating pregnant women

<table>
<thead>
<tr>
<th>Unhealthy eating components</th>
<th>Frequency intakea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
</tr>
<tr>
<td>Sugar (n=93)</td>
<td>16.15</td>
</tr>
<tr>
<td>Processed meat (n=94)</td>
<td>2.5</td>
</tr>
<tr>
<td>Junk food (n=94)</td>
<td>6.42</td>
</tr>
</tbody>
</table>

- Frequencies were not normally distributed and median (range) reported

5.5.3. Infant feeding practice

Of 129 women that received the MD questionnaire, 79 completed the questionnaire (61.2%). One child was extremely premature, born at 24-25 gestation weeks and the MD was not posted. Weight of babies at 2-3 months of age was within the normal range, whereas age of the introduction of formula was not normally distributed. Mean weight of babies at 2-3 months was 5.51kg. Details of introduction of drinks and solids into the diet of babies and their feeding characteristics are presented in Table 5.7. There were missing replies to weight of babies and a few other questions. Mean age of babies at the time of completion the MD questionnaire was 2.7 months. Where the distributions of continuous data were within normal range, mean and SD is reported.
and otherwise, median and range reported. Infant formulas consumed more commonly were Aptamil, C&G and SMA and the specialised formulas, used by three infants, were Nutramigen and SMA staydown. Water was the only drink reported to have been introduced by 11 participating women (introduced at <1 month one baby, 1-2 months five babies, 2-3 months four babies and >3 months one baby). Seven mothers reported that they added items into their baby’s bottle and these were listed as Gaviscon (six babies) and Gripe water (one baby). Only two women stated that they had introduced solids into the diet of their babies and specified these as baby rice, pureed vegetable and bean sauce for the two babies. Expressed breast milk was introduced by nine mothers [with median (range) 300.00 (90.00-738.74ml)].
Table 5.7. Baby weight measurements at 2-3 and 6 months follow-ups and feeding characteristics of babies at 2-3 months

<table>
<thead>
<tr>
<th></th>
<th>MD responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baby weight at 2 months (n=75)</td>
<td>Mean kg (SD)</td>
</tr>
<tr>
<td>Weight/age at 2 months (SD units), whole sample (n=73)</td>
<td>5.51 (0.96)</td>
</tr>
<tr>
<td>Weight/age at 2 months (SD units), term babies (n=64)</td>
<td>-0.17 (1.11)</td>
</tr>
<tr>
<td>Baby weight at 6 months (n=73)</td>
<td>Mean kg (SD)</td>
</tr>
<tr>
<td>Weight/age at 6 months, whole sample (n=71) (SD units)</td>
<td>7.63 (1.35)</td>
</tr>
<tr>
<td>Weight/age at 6 months, term babies (n=66)</td>
<td>0.102 (1.67)</td>
</tr>
</tbody>
</table>

Introduction of formula (%)
- Yes: 45 (57.0)
- No: 34 (43.0)

Age at 1st introduction of formula (days) (n=44)
- Median (range): 3 (1-91)

Type of formula
- Regular: 42 (93.3)
- Specialised: 3 (6.7)

Introduction of drinks (n=79)
- Yes: 11 (13.9)
- No: 68 (86.1)

Adding anything to baby bottle (n=78)
- Yes: 7 (9.0)
- No: 71 (91.0)

Introduction of solids (n=78)
- Yes: 2 (2.6)
- No: 76 (97.4)

Current feeding practice of infant
- Exclusive breast-feeding: 35 (44.3)
- Exclusive formula-feed: 32 (40.5)
- Partially breast-feed: 12 (15.2)

Median volume of formula (ml/day)
- Median (range): 781.36 (0.50-1086.79)

Median number of formula feed (day)
- Median (range): 5.25 (0.25-9)

Median duration of breast-feeding (minute) per day
- Median (range): 116.25 (45-324)

Median number of breast-feeding per day
- Median (range): 8.75 (4.00-15.25)

Median volume of breast milk intake (ml) (based on infant’s age)
- Median (range): 850 (345.26-875.00)

Median volume of milk intake (ml) (breast/formula/both)
- Median (range): 850 (564.46-1086.79)

Data for 4 babies were missing
Relevant data for calculation of weight/age were not available for 2 infants
* Collected at 2nd follow-up
** Relevant data for calculation of weight/age were not available for 2 infants
*** Relevant data for calculation of weight/age were not available for 9 infants

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5.5.4. Main outcome measures

5.5.4.1. Correlation between FFQ-P food components and their predictive effect on infant birth weight Z-score

The correlation between AHEI-P food components is shown in Table 5.8. The vegetable/fruit component was found to be significantly correlated with the iron-rich, fibre and dairy components. Iron-rich foods, dairy and fibre components were also positively correlated to each other, whereas all were weakly inversely correlated with the fat component. The fish component was not correlated to any of the other food components and Vitamin D intake had a negative weak correlation with all the food components, except for the vegetable/fruit and fibre components.

Sugar intake and unhealthy junk food consumption were significantly correlated, and processed meat was also significantly correlated with sugar. All the unhealthy eating habits were negatively correlated with the total AHEI-P score and a significant correlation was found for the junk food component. Correlation of unhealthy eating habits and total AHEI-P score is shown in Table 5.9.

Using the one-way ANOVA analysis, the association between unhealthy eating habits as well as AHEI-P score and question of “how much attention do you pay to your diet in term of healthy eating?” was also investigated. The replies to this question were: “very little”, “somewhat” and “a great deal”. Women who responded that attention to their diet was “a great deal” scored lower for the sugar consumption compared to the “very little” response only (p=0.04). There were no significant differences between the responses to this question with “processed meat” and “junk food” unhealthy eating habits. In addition, mean score of AHEI-P for women that responded “a great deal” were significantly higher than the replies specified as “very little” (p=0.04) and “somewhat” (p=0.005).
Table 5.8. Correlation of AHEI-P food components to each other

<table>
<thead>
<tr>
<th></th>
<th>Vegetable/fruit</th>
<th>Iron-rich foods</th>
<th>Dairy</th>
<th>Fibre</th>
<th>Fat</th>
<th>Fish</th>
<th>Vitamin D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegetable/fruit</td>
<td>1</td>
<td>.33**</td>
<td>.21*</td>
<td>.32**</td>
<td>.05</td>
<td>.15</td>
<td>.002</td>
</tr>
<tr>
<td>Iron-rich foods</td>
<td></td>
<td></td>
<td>.01</td>
<td>.03</td>
<td>.001</td>
<td>.62</td>
<td>.14</td>
</tr>
<tr>
<td></td>
<td>.33**</td>
<td>1</td>
<td>.34**</td>
<td>.37**</td>
<td>-20*</td>
<td>.01</td>
<td>-.01</td>
</tr>
<tr>
<td></td>
<td>.01</td>
<td>.01</td>
<td>.000</td>
<td>.04</td>
<td>.87</td>
<td>.86</td>
<td></td>
</tr>
<tr>
<td>Dairy</td>
<td>.21*</td>
<td>.34**</td>
<td>1</td>
<td>.15</td>
<td>-.01</td>
<td>.04</td>
<td>-.04</td>
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<tr>
<td></td>
<td>.03</td>
<td>.001</td>
<td>.13</td>
<td>.86</td>
<td>.67</td>
<td>.69</td>
<td></td>
</tr>
<tr>
<td>Fibre</td>
<td>.32**</td>
<td>.37**</td>
<td>.15</td>
<td>1</td>
<td>-25*</td>
<td>.09</td>
<td>.09</td>
</tr>
<tr>
<td></td>
<td>.01</td>
<td>.000</td>
<td>.13</td>
<td>.01</td>
<td>.37</td>
<td>.37</td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td>.05</td>
<td>-.20*</td>
<td>-.01</td>
<td>-.25*</td>
<td>1</td>
<td>.001</td>
<td>-.05</td>
</tr>
<tr>
<td></td>
<td>.62</td>
<td>.04</td>
<td>.86</td>
<td>.01</td>
<td>.98</td>
<td>.62</td>
<td></td>
</tr>
<tr>
<td>Fish</td>
<td>.15</td>
<td>.01</td>
<td>.04</td>
<td>.09</td>
<td>.001</td>
<td>1</td>
<td>-.06</td>
</tr>
<tr>
<td></td>
<td>.14</td>
<td>.87</td>
<td>.67</td>
<td>.37</td>
<td>.98</td>
<td>.52</td>
<td></td>
</tr>
<tr>
<td>Vitamin D</td>
<td>.002</td>
<td>-.01</td>
<td>-.04</td>
<td>.09</td>
<td>-.05</td>
<td>-.06</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>.98</td>
<td>.86</td>
<td>.69</td>
<td>.37</td>
<td>.62</td>
<td>.52</td>
<td></td>
</tr>
</tbody>
</table>

1st and 2nd rows in each cell represent Spearman Rank Order correlation coefficient and p value
**Correlation is significant at the 0.01 level (2-tailed)
*Correlation is significant at the 0.05 level (2-tailed)
Table 5.9. Correlation of unhealthy eating habits and total AHEI-P scores

<table>
<thead>
<tr>
<th></th>
<th>Sugar</th>
<th>Processed meat</th>
<th>Junk food</th>
<th>Total AHEI-P score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugar</td>
<td>1</td>
<td>.20*</td>
<td>.37**</td>
<td>-.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td>.04</td>
<td>.000</td>
<td>.45</td>
</tr>
<tr>
<td>Processed meat</td>
<td>.20*</td>
<td>1</td>
<td>.04</td>
<td>-.009</td>
</tr>
<tr>
<td></td>
<td>.04</td>
<td>.69</td>
<td>93</td>
<td></td>
</tr>
<tr>
<td>Junk food</td>
<td>.37**</td>
<td>.04</td>
<td>1</td>
<td>-.21*</td>
</tr>
<tr>
<td></td>
<td>.000</td>
<td>.69</td>
<td>.03</td>
<td></td>
</tr>
<tr>
<td>Total AHEI-P score</td>
<td>-.07</td>
<td>-.009</td>
<td>-.21*</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>.45</td>
<td>.93</td>
<td>.03</td>
<td></td>
</tr>
</tbody>
</table>

*Correlation is significant at the 0.05 level (2-tailed)  **Correlation is significant at the 0.01 level (2-tailed)
Multiple linear regression analyses, using a standard regression process, were undertaken to investigate whether maternal dietary habits during pregnancy predict the infant birth weight Z-score. The model was checked for the required assumptions and no violation for normality, linearity, multicollinearity and homoscedasticity were found. In the final model, 6% of the variance in infant birth weight Z-score could be explained (R=0.06, SE=0.85).

The results indicated that maternal sugar consumption contributed the most to infant birth weight Z-score ($\beta$=-0.01, p=0.007). Table 5.10 shows the details of the associations. These results did not change when the analysis was restricted to term babies (n=82).

Table 5.10. Linear regression model for the association between AHEI-P and birth weight Z-score

<table>
<thead>
<tr>
<th>The model (n=91)</th>
<th>$\beta$ (SE)</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lower bound</td>
<td>Upper bound</td>
</tr>
<tr>
<td>AHEI-P score</td>
<td>0.01 (0.01)</td>
<td>-0.007</td>
<td>0.03</td>
</tr>
<tr>
<td>Sugar consumption</td>
<td>-0.01 (0.007)</td>
<td>-0.03</td>
<td>-0.005</td>
</tr>
<tr>
<td>Processed meat</td>
<td>0.03 (0.04)</td>
<td>-0.04</td>
<td>0.11</td>
</tr>
<tr>
<td>Junk food</td>
<td>0.01 (0.01)</td>
<td>-0.02</td>
<td>0.04</td>
</tr>
</tbody>
</table>

There was one outlier in baby’s birth weight Z-score (>3) and excluding that baby from the analysis the effect of maternal sugar consumption during pregnancy on baby’s birth weight Z-score became non-significant (p=0.10).

5.5.4.2. Predictors of baby weight Z-score at 2-3 months of age

To determine whether the type of milk infants consumed (breast, formula and partial) at 2-3 months of age is associated with weight/age Z-scores of babies’ at the two follow-up time points, a one-way ANOVA was conducted (Table 5.11). There was no significant difference between the infant weight/age at 2-3 months age and the type of milk fed between groups or within groups (p=0.24) and the results remained non-significant when only the term babies at birth were included in the analysis (n=64). There was one outlier in baby weight at 2 months (Z-score >3) and when excluding that baby from the analysis the results remained non-significant.

As shown in Table 5.11, a significant difference was found for the weight/age Z-score at 6 months (p=0.01) and the effect remained significant when only term-babies at birth were included in the analysis (p=0.002). The detailed descriptive data (LSD test) indicated partially breast-fed infants had lower weight/6months Z-score than both breastfed and formula-fed infants. When the outliers were excluded from the analysis (Z-score <-3 and >3, two and one cases respectively), the results remained significant (n=59, p=0.01).
Table 5.11. Associations between infant feeding behaviours at 2-3 months and weight/age Z-scores at 2-3 and 6 months

<table>
<thead>
<tr>
<th></th>
<th>Weight/2 months Z-score (n=73)</th>
<th>P value</th>
<th>Weight/6 months Z-score (n=62)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD units)</td>
<td></td>
<td>Mean (SD units)</td>
<td></td>
</tr>
<tr>
<td>Exclusively BF</td>
<td>0.006 (1.09) n=33</td>
<td>0.24</td>
<td>-0.09 (1.26) n=28</td>
<td>0.01</td>
</tr>
<tr>
<td>Exclusively formula feeding</td>
<td>-0.206 (1.02) n=29</td>
<td></td>
<td>0.20 (1.37) n=25</td>
<td></td>
</tr>
<tr>
<td>Partially BF</td>
<td>-0.64 (1.33) n=11</td>
<td></td>
<td>-1.24 (0.92) n=9</td>
<td></td>
</tr>
</tbody>
</table>

Multiple linear regression analysis, using a standard regression process, was undertaken to investigate whether maternal dietary habits during pregnancy as well as infant milk feed type predict the baby weight Z-score at 2-3 months of age. There was no violation of the assumptions of normality, linearity, multicollinearity and homoscedasticity. In the final model, 3.6% of the variance in infant weight at 2 months Z-score could be explained (R=0.036, SE=1.00). None of the factors included were significant predictors of the weight Z-score at 2-3 months of age (Table 5.12).

Table 5.12. Linear regression model for predictors of baby weight at 2 months Z-score

<table>
<thead>
<tr>
<th>The model (n=65)</th>
<th>β (SE)</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infant feeding</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast-fed</td>
<td>1 (ref)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formula fed</td>
<td>-0.12 (0.28)</td>
<td>-0.68</td>
<td>0.44</td>
</tr>
<tr>
<td>Partially breast-fed</td>
<td>-0.74 (0.40)</td>
<td>-1.55</td>
<td>0.06</td>
</tr>
<tr>
<td>AHEI-P score</td>
<td>-0.01 (0.01)</td>
<td>-0.04</td>
<td>0.01</td>
</tr>
<tr>
<td>Sugar consumption</td>
<td>0.006 (0.009)</td>
<td>-0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>Processed meat</td>
<td>0.05 (0.05)</td>
<td>-0.06</td>
<td>0.16</td>
</tr>
<tr>
<td>Junk food</td>
<td>-0.04 (0.02)</td>
<td>-0.09</td>
<td>0.009</td>
</tr>
</tbody>
</table>

When excluding the one outlier from the regression analysis (Z-score >-3), a significant association was found for partially breast-fed infants indicating that these babies had lower weight at 2 months Z-score (p=0.04).

Looking at breast-feeding infants only, the model was also adjusted for the mean duration and number of breast-feeding per day (n=32). None of the covariates contributed to the models.

When the model only included formula-fed infants, excluding the outlier on baby’s weight at 2 months Z-score, the mean volume and number of formula feedings per day were added into the model (n=25). The model explained 54% of the variances (R=0.54, SE=0.70). Mean number of formula feedings per day in
addition to AHEI-P score and junk food made a significant contribution to the models. Details are shown in Table 5.13.

Table 5.13. Linear regression model for predictors of baby weight at 2 months Z-score in formula-fed babies

<table>
<thead>
<tr>
<th>The model (n=25)</th>
<th>β (SE)</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean volume of formula-feeds per day (ml)</td>
<td>0.002 (0.001)</td>
<td>0.000 - 0.005</td>
<td>0.09</td>
</tr>
<tr>
<td>Mean number of formula-feeds per day</td>
<td>-0.28 (0.12)</td>
<td>-0.54 - -0.02</td>
<td>0.03</td>
</tr>
<tr>
<td>AHEI-P score</td>
<td>-0.04 (0.01)</td>
<td>-0.08 - -0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Sugar consumption</td>
<td>0.007 (0.01)</td>
<td>-0.01 - 0.02</td>
<td>0.49</td>
</tr>
<tr>
<td>Processed meat</td>
<td>-0.08 (0.06)</td>
<td>-0.22 - 0.05</td>
<td>0.22</td>
</tr>
<tr>
<td>Junk food</td>
<td>-0.08 (0.03)</td>
<td>-0.15 - -0.003</td>
<td>0.04</td>
</tr>
</tbody>
</table>

When age of introduction of formula was added into the above model (n=24), no significant association was observed for this variable and the effect of maternal AHEI-P score also became non-significant (p=0.06).

5.5.4.3. Predictors of baby weight Z-score at 6 months of age

Multiple linear regression analyses, using a standard regression process were undertaken to investigate whether maternal dietary habits during pregnancy as well as infant’s milk-fed type at 2 months and introduction of solids predict the baby’s weight Z-score at 6 months of age. No violations of the assumptions of normality, linearity, multicollinearity and homoscedasticity were found. In the final model, 16% of the variance in infant’s birth weight Z-score could be explained (R=0.16, SE=1.21). Frequency of sugar consumption per week was the only factor that significantly contributed to the model (Table 5.14).

Table 5.14. Linear regression model for predictors of baby weight at 6 months Z-score

<table>
<thead>
<tr>
<th>The model (n=43)</th>
<th>β (SE)</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infant feeding</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast-fed</td>
<td>1 (ref)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formula fed</td>
<td>0.58 (0.41)</td>
<td>-0.25 - 1.42</td>
<td>0.16</td>
</tr>
<tr>
<td>Partially breast-fed</td>
<td>-0.77 (0.61)</td>
<td>-2.01 - 0.46</td>
<td>0.21</td>
</tr>
<tr>
<td>AHEI-P score</td>
<td>-0.02 (0.02)</td>
<td>-0.06 - 0.01</td>
<td>0.20</td>
</tr>
<tr>
<td>Sugar consumption</td>
<td>-0.03 (0.01)</td>
<td>-0.07 - -0.002</td>
<td>0.04</td>
</tr>
<tr>
<td>Processed meat</td>
<td>-0.07 (0.09)</td>
<td>-0.27 - 0.11</td>
<td>0.42</td>
</tr>
<tr>
<td>Junk food</td>
<td>-0.04 (0.03)</td>
<td>-0.11 - 0.03</td>
<td>0.25</td>
</tr>
<tr>
<td>Introduction of solids (weeks)</td>
<td>-0.02 (0.04)</td>
<td>-0.12 - 0.07</td>
<td>0.64</td>
</tr>
</tbody>
</table>
When excluding the outliers from the regression analysis (Z-score < -3 and > 3), the significant effect for maternal sugar consumption on weight at 6 months Z-score was weakened (n=42, p=0.07).

Subgroup analyses on breast and formula feeding babies were not conducted since the number of infants within each group were small (n=21 and 17 respectively).

The association between types of milk consumed at 6 months (breast, formula and partial-fed) and weight at 6 months Z-score was also investigated in ANOVA analysis and no significant differences were found (p=0.13).

5.6. Discussion

The primary objective of this cohort study was to investigate the associations between maternal dietary intake during pregnancy and infant feeding practices in early life with infant weight Z-scores at birth, 2-3 and 6 months of age. Overall, the quality of maternal diet in this sample of pregnant women was suboptimal. Partially breast-fed infants had lower weight at 6 months Z-score (ANOVA analysis, Table 5.11) and also, a lower weight Z-score at 2 months in an adjusted model when an outlier was excluded. In sub-group regression analysis, the number of formula feeds together with AHEI-P score and consumption of junk foods in pregnancy predicted the baby weight Z-score at 2 months. Furthermore, inverse associations were found between maternal sugar consumption during pregnancy with weight/age at birth and 6 months of age, although these effects became non-significant when excluding the outliers on baby weight from the models. The following sections will discuss these results.

5.6.1. Maternal diet during pregnancy

Mean total score of the AHEI-P in pregnant women was low (mean=33.60/70, SD=8.93), meaning that the quality of diet in women enrolled in this study was poor. The vegetable/fruit component was the most highly scored food group (mean score=8.87/10) followed by fat (from unsaturated sources) (mean score=7.36/10). The most poorly scored food component was fibre (mean score=1.99/10) and overall, scores for the other food components were low (Table 5.5). The frequency of intakes of unhealthy items (sugar, processed meat and junk food) were also relatively high (Table 5.6) and these were negatively correlated with the AHEI-P score, which was only significant for the junk food component. Significant correlations were identified between vegetable/fruit, iron-rich foods, dairy and fibre components; however the dairy and fibre components were not correlated. Also negative or no correlations were found between these components and fat, fish and Vitamin D components. One reason could be that the scores for the latter components were calculated differently since these were extracted from open questions and
data on frequency consumption were not included. The fat component also showed negative significant correlations with iron-rich foods and fibre components. The fact that there are no clear guidelines for the consumption of fat but only for the type (unsaturated vs. saturated fats) pinpoints the difficulties regarding measurement of fat intake. Also, there are a lot of confusing messages from the media about the healthiest types of oil/fat to consume. For example, while coconut oil is a rich source of saturated fat, it is usually promoted as a healthy oil/fat. Inadequate consumption of Vitamin D from supplements and omega-3 from foods i.e. oily fish was also a concern in this study sample, particularly because women might limit consumption of fish during pregnancy due to pregnancy sickness and safety reasons.

Several lines of research have investigated the adequacy of maternal diet during pregnancy, using either diverse healthy eating index scores and dietary patterns or quantifying the intake of foods/nutrients by serving sizes. The results from this study are in line with previous research indicating that pregnant women do not meet the national recommendations outlined in the dietary guidelines and generally have a suboptimal quality of diet for many nutrients (Blumfield, Hure, MacDonald-Wicks, Smith, & Collins, 2012; Malek, Umberger, Makrides, & Zhou, 2015; Morton et al., 2014). Also consistent with this study, it has been shown that usually adherence of women to consumption of fruit and vegetable in pregnancy is favourable (Skreden, Bere, Sagedal, 2017), whilst intake of fibre, carbohydrates and PUFAs are commonly lower than the recommendations (Blumfield, et al., 2012).

It can be hypothesised that many women do not sustain a healthy diet prior to pregnancy and subsequently maintain the same nutritional behaviour during pregnancy. It might be that women do not have accurate knowledge of relevant dietary guidelines and hold misbeliefs and incomplete knowledge leading them to make inappropriate food choices at reproductive age. In support of this hypothesis, previous research from longitudinal studies reported that overall women maintain their dietary patterns from pre-pregnancy throughout pregnancy, with little changes except for the consumption of some nutrients (Crozier, Robinson, Godfrey, Cooper, & Inskip, 2009). For example, it has been found that intake of fruit is increased during pregnancy although there are mixed reports for the consumption of vegetables (Chen et al., 2013; Crozier, et al., 2009; Pinto, Barros, Dos, & Silva, 2009).

Diet in pregnancy has a crucial role for the health of both mother and foetus. Evidence indicates that the nutritional state of women prior to pregnancy together with nutrition during pregnancy may influence both pregnancy and foetal outcomes (Gluckman, 2008; Inskip et al., 2014; Martin et al., 2016). Generally speaking, diet is a complex behaviour that is affected by a number of factors. Systematic reviews from observational and qualitative studies have specified a number of factors including pregnancy-related
(parity, sickness), socio-demographic (age, educational level, income and co-habiting), psychological (stress, anxiety, depression), environmental (social, food environment) in addition to self-efficacy beliefs and nutrition knowledge that could influence dietary behaviours of women prior and during pregnancy (Doyle, Borrmann, Grosser, Razum, & Spallek, 2016; Malek, Umberger, Zhou, & Makrides, 2015). In the current study, the statistical analyses regarding the influential factors on quality of maternal diet e.g. demographic characteristics were not conducted since it was beyond the scope of this thesis.

To conclude, the fact that the findings of this study and others indicate that in general pregnant women fail to meet the key food and nutrient targets highlights the importance of community interventions in order to improve knowledge of nutritional choices and healthy eating during pregnancy. It is also important to study the underlying factors of how the acquired knowledge could be translated into behaviour at the population level.

5.6.2. Infant weight and feeding characteristics

Overall, most babies were born at full term (83.6%) and the mean birth weight was 3.32kg which is in line with national data of mean birth weight 3kg (McAndrew et al., 2010). The birth weight Z-score for term babies was also broadly average (SD units=0.03). Most babies were breast-fed (74.8%) on their first day of birth, and the rest were either bottle-fed (20%) or fed with both methods (4.4%). The median age of introduction of formula was 3 days (range=1-91). At two months of age, 44.3% of babies were exclusively breast-fed with 40.5 and 15.2% being exclusively formula-fed and partially breast-fed, respectively. McAndrew and colleagues (2010) reported that the current initiation rate of breast-feeding in the UK is 81%, and the prevalence rate declines to 55 and 34% at six weeks and six months respectively. Nationally, 67% of infants are being introduced to formula between 4-10 weeks at least once, and approximately half of the infants are exclusively formula-fed (46%). Overall, data on infant feeding practices in the current study compares well to national data.

In the current study, ANOVA analysis for type of milk consumed at 2 months of age showed that partially breast-fed babies weighed significantly less than exclusive breast and formula-fed infants at 6 months (Table 5.11). However, the analysis for milk consumed (breast, formula, partial-fed) at 6 months of age did not show an association with weight at 6 months Z-score. The adjusted regression model, excluding the outlier on baby weight at 2 months of age, showed partial breast-fed babies had a lower mean weight at 2 months Z-score (p=0.04).
As described in Chapter 2 section 2.9.3, the association between breast-feeding and childhood obesity has been assessed in numerous studies. Large discrepancies exist between the conducted studies in terms of measurement of breast-feeding as well as obesity, and systematic reviews have been conducted to infer a conclusive result. An overview of systematic reviews by Patro-Goląb and colleagues (2016) concluded that any duration of breast-feeding could reduce the risk of overweight and obesity in children by 13%, while exclusive breast-feeding does not protect against childhood obesity. The results from this study are consistent with this overview of systematic reviews, although the current study only examined "weight for age" in very early life as a growth measure and further follow-ups of these babies could provide data on later risk of obesity. The non-significant findings between the type of milk consumed at 6 months and weight at 6 months Z-score could be because the totality of diet at 6 months of age is affected by other factors such as starting weaning and volume of milk consumed. The study might have also been underpowered to detect any associations.

Moreover, subgroup analysis for the type of milk feeding in this PhD study showed there was an inverse significant association between number of formula feedings with weight at 2 months Z-score (Table 5.13), indicating that perhaps babies were fed with a higher volume of formula per feed. Similarly, combined pooled data from a large study on the West Australian Pregnancy Cohort (Raine) and 3 European cohort studies showed that introducing formula earlier than 3 months of age was significantly associated with rapid growth patterns in infants by 6 years and BMI trajectories by 20 years (Rzehak et al., 2017). Altered body composition of formula-fed babies compared to breast-feeding babies is also established in a systematic review and meta-analysis (Gale et al., 2012) that is characterised with a transient lower fat mass and a higher fat-free mass from 3-6 months of age. Overall, human breast milk contains growth hormones and bioactive factors, and infants receive less energy per volume. The main differentiating factor between formula and breast milk appears to be the protein content and thus, formula feeding could surge the number and size of adipocytes in infants (Brands, Demmelmaier, & Koletzko, 2014; Ziegler, 2006). Furthermore, a systematic review of RCTs reported that consumption of formula milk with lower protein concentration could have transient effects on baby growth from 3-12 months age and concluded the available evidence is limited for assessing the effects of protein concentration in infant formulas on later risk of obesity in children (Patro-Goląb et al., 2016). Subsequently, formula feeding remains a primary risk factor for early childhood obesity.

Ideally, the infant feeding type in the current study could be grouped: exclusively breast-fed (BF), dominantly BF, partially BF and exclusively formula-fed that would provide more reliable data for statistical purposes. However, the sample in this study was not sufficiently powered to distinguish the
differences using this grouping with the small number of infants within each group. In addition, in this PhD study, “weight Z-scores” was the only parameter for quantifying growth in babies, since measures on baby height was recorded for only a small percentage of babies in the follow-up questionnaires, and therefore it was not possible to calculate BMI.

5.6.3. Associations between maternal diet during pregnancy and infant weight/age Z-scores

The quality of maternal diet during pregnancy measured by AHEI score in this study was not associated in the adjusted models with weight Z-score either at birth or baby follow-ups at 2 and 6 months. Subgroup analysis on formula-fed babies however showed inverse associations between maternal AHEI-P score and junk food consumption during pregnancy with weight at 2 months Z-score, so that babies of mothers who ate more junk food and better met the UK food guidelines during pregnancy weighed less at 2 months. In addition, babies of mothers who consumed more sugar in pregnancy had a lower birth weight Z-score and the effect persisted by 6 months of age, although the effect at both time points became non-significant by excluding the outliers on baby weight from the models. The inverse association observed between AHEI score and weight at 2 months Z-score could be because of the small sample included in the current study. Further research with a larger sample could replicate these findings.

Studies assessing the impact of maternal diet during pregnancy on birth weight and/or SGA or the risk of obesity later in life have either used a priori or a posteriori approach. A priori approach measures the quality of diet using national or international recommendations, as used in the current study and is shown to have a good generalisability across different cohorts (Liese et al., 2015). A posteriori approach is data-driven using different statistical methods for extracting dietary patterns and is highly dependent on the population under study. A few studies that measured a diet quality index in pregnant women have shown positive associations between higher diet quality scores and growth parameters at birth i.e. weight and length (Ferland & Turgeon O’Brien, 2003; Rodríguez-Bernal et al., 2010). A recent cohort of 1,079 pregnant women assessed the effect of quality of diet, via repeated 24-hour recall on neonatal adiposity and showed HEI score ≤57 increased percentage fat mass and fat mass at birth (Shapiro et al., 2016). Moreover, the Norwegian Mother and Child Cohort Study of 62,494 term singleton pregnancies has established evidence that consumption of rapidly absorbed sugar from sugar-sweetened carbonated soft drinks had inverse associations with birth weight in normal pregnancies (Grundt, Eide, Brantsæter, Haugen, & Markestad, 2016). Other research assessing the effect of maternal diet during pregnancy on later risk of obesity has shown contradictory results. One study from China established a link between a dietary pattern higher in intake of vegetables/fruit and lower in fast foods, with reduced risk of child adiposity at 4.5 years (Chen et al., 2017), a cohort from Spain with maternal adherence to a
Mediterranean diet was not associated with childhood overweight at 4 years but lower waist circumference (Fernandez-Barres et al., 2016). In the current study, the lack of an association between AHEI score and weight Z-scores at birth and follow-ups could be because the study was not sufficiently powered to detect the associations. Also, it was not possible to consider the role of other underlying confounders such as maternal pre-pregnancy BMI, GDM or weight gain during pregnancy in the regression models due to limitations in the collected data; however, the models were adjusted for unhealthy eating habits during pregnancy, and weight for age Z-scores were also calculated. The significant associations observed for maternal consumption of sugar at birth and 6 months and also for the AHEI-P score and junk food (for formula-fed babies) in the current study, still provide some evidence for the importance of quality of maternal diet during pregnancy and warrants further research within this under investigated population. It is documented that environmental factors e.g. poor quality of maternal diet in pregnancy has damaging effects on placental vascular network and possibly the state of oxidative stress leading to placental dysfunction that consequently contributes to abnormal foetal development (Pereira et al., 2015).

A number of studies have assessed the impact of dietary patterns during pregnancy on birth weight or SGA and their findings are comparable with the current study. A large study from the UK on the Avon longitudinal data found five dietary patterns in pregnant women: health conscious, traditional, processed, confectionary and vegetarian (Northstone, Emmett, & Rogers, 2008), and reported a health conscious diet pattern was associated with better birth weight. A review of seven studies from high-income countries (the UK, US, Denmark, New Zealand, Netherlands and Japan) on dietary patterns in pregnancy and birth weight indicated data were collected at different time points from throughout pregnancy to years after birth (Kjøllesdal & Ottesen, 2014). Dietary patterns were explored using principal component analysis (5 studies), and also cluster analysis and logistic regression (each in one study) and between one and seven dietary patterns were reported in the studies. Across studies, the diet patterns positively associated with birth weight were: “nutrient dense, protein rich, health conscious and Mediterranean” whereas “western, processed, vegetarian, transitional and wheat products” were negatively associated with birth weight. Further studies have provided evidence that a “varied diet” in China (Lu et al., 2016), a “vegetable, fruit and white rice” pattern in Singapore (Chia et al., 2016), a “health conscious diet” in Ghana (Abubakari & Jahn, 2016) and a pattern characterised by intake of “eggs, starchy vegetables and non-whole grains” in the US (Starling et al., 2017) were associated with either birth weight size or newborn adiposity. Collectively, it should be stressed that although the dietary patterns across various studies were termed differently, common features can be found between the patterns. For example, high consumption of vegetables, fruit and dairy products are common characteristics of dietary patterns associated with higher
birth weight and comparably, patterns linked with low birth weight are typically characterised by elevated loadings of processed, high-fat meat, fats and oils and sugar rich products. In summary, data from both diet quality and dietary patterns research in pregnancy signifies the importance of maternal diet on infant growth parameters and risk of overweight/obesity later in life.

5.6.4. Strengths
The sample of pregnant women enrolled in this cohort study is representative of the Portsmouth population in terms of ethnic group profile (82.1% white British), although the proportion of married women and who owned a property was higher than the Portsmouth figures (Research and Intelligence, 2013). A particular strength of this study is that the data was collected prospectively which would minimise the recall bias and there was a good response rate (91.4 and 61.2% to FFQ-P and MD, respectively). Diet in pregnant women was assessed as a whole where quality of diet was measured by AHEI score, reflecting consumption of actual foods and food groups similar to other studies. It can be argued that whilst measuring grams of foods or food groups could facilitate national and/or international comparisons of dietary intakes, this approach fails to evaluate the appropriateness of dietary guidelines in relation to population group intake. Moreover this is the first report of its kind from the city of Portsmouth, although one might argue that the sample is small and might not be nationally representative. All questionnaires were also specific to the study groups and validated, and the coding, analysis and interpretation of results conducted by the same researcher to minimise the effect of researcher error.

5.6.5. Limitations
The present study did have a number of limitations. The key one is the small sample included in this cohort of pregnant women, thus limiting the generalisability of the results. There are some limitations for the FFQ-P used since the replies rely on respondent’s memory, and therefore it is subject to recall bias. The frequency of food consumption could have also been overestimated and specifically for the healthy food items. Also, the nature of data collected did not allow us to calculate the frequency of consumption of fat, Vitamin D and omega-3 from vitamins with the same method used for the other food components. Moreover, consumption of sugar in pregnancy was calculated for the sugary items in the questionnaire, a mix of drinks and chocolate and as an example, not particularly for artificially sugar-sweetened beverages, so this might limit the comparability of these data with other research.

With regards to the MD questionnaire, the nutritional intakes were estimated and not validated against any biomarkers. In addition, it takes a number of days to complete and therefore this demands a level of responsibility for mothers which could have had the potential to minimise the response rate. The unavailability of other growth parameters and adiposity measures in infants along with the short-term
follow-up might also be a limitation of this study. In addition, the regression models were relatively weak and data on other maternal-related risk factors were not available i.e. pre-conception overweight/obesity, gestational weight gain and diabetes. However there were not sufficient resources to address these for this PhD study.

5.6.6. Conclusion

Both maternal diet during pregnancy and early feeding practices in infants could have life-long lasting effects on weight outcomes in infants. Further larger studies could clarify whether these should be targeted in public health policies for early prevention of childhood obesity.
Chapter 6: Relationship between family history of allergy, quality of maternal diet during pregnancy, infant diet and the development of allergic outcomes in children by 6 months of age

6.1. Overview of the chapter

This chapter examines the relationship between family history of allergy, quality of maternal diet during pregnancy, infant diet and the development of allergic outcomes in children by 6 months of age. Parents/guardians were asked to complete a validated questionnaire of Food Frequency Questionnaire (FFQ) when their child was 6 months of age. The questionnaire used will be described and its choice justified. Symptoms of allergy in children were also captured at 6 months and they are considered in relation to infant dietary intake as well as family history of allergy and quality of maternal diet during pregnancy. The findings are discussed in terms of their contribution to the existing evidence base on early risk factors of allergy.

6.2. Objectives

a. To describe family history of allergies in children and its association with development of allergic symptoms at 6 months of age
b. To determine the effect of maternal dietary intake during pregnancy, measured by AHEI score and the development of allergic symptoms in children at 6 months of age
c. To determine infant feeding practices (breast vs. formula fed) at 6 months and its association with allergic symptoms
d. To investigate the association between introduction of key allergenic foods in infant diet and allergic symptoms at 6 months of age
e. To investigate the association between other dietary intakes and allergic symptoms at 6 months of age
f. To determine if any of the factors listed in a-e are predictors of allergic outcomes at 6 months of age

6.3. Rationale for choice of questionnaire

Questionnaires were selected if they had been validated in the target group and they were relevant for the study age group. In addition, the time needed to complete the questionnaires was considered.
6.3.1. Measuring infant dietary intake

As already outlined in Chapter 5 (section 5.3.1), measuring dietary intake or dietary variety is defined by a simple count of foods or food groups consumed over a given reference period (Ruel, 2003). In this study, dietary intake of infants at 6 months was quantified by the number of foods using a validated FFQ. A number of specifically designed infant FFQs have been developed, as summarised in a systematic review (Ortiz-Andrellucchi et al., 2009). The questionnaire administered in this study (appendix 6.1, section 3.16) was an amended version of the Southampton Women’s Survey FFQ at 6 months (Marriott et al., 2008). This particular FFQ was chosen since it was validated in a group of 6-month-old infants against a four-day weighed food diary in a geographical population similar to this target study population. The original questionnaire was semi-quantitative, asking portion sizes and frequency intake of foods. For this study, the portion sizes were removed since the nutritional information was not being assessed and therefore, the FFQ in this study only asked for the frequency of intake. The questionnaire consisted of a list of 38 foods and drinks, divided into subcategories. The subcategories of food and drinks were: ready-made baby foods (12 foods), starchy foods (eight foods), vegetables (two items), fruit (three items), yogurt and fromage frais (one item), meat and fish (two foods) and non-water drinks (nine items) and water. The frequency of consumption over the previous month of each food and drink was recorded using a multiple response grid. The frequency options were: never, 1-3 times/month, once/week, 2 times/week, 3 times/week, 4 times/week, 5 times/week, 6 times/week, 7 times/week and more than once a day. Parents were asked to indicate the frequency of each item by ticking the appropriate box. Parents were also asked about the type of milk the infant was consuming and if formula-fed, to specify timing of introduction and its type in addition to the age of introduction of solids.

6.3.2. Identification of allergic symptoms and consumption of other allergenic foods

Allergic symptoms at 6 months were established using the most widely utilised self-reported validated tool- the International Study of Asthma and Allergies in Childhood questionnaire (ISAAC) (Asher et al., 1995). Since there is no gold standard for the classification of allergic diseases at a very young age, a combination of symptoms were used for identification of allergic symptoms. For the purpose of this study, the ISAAC questionnaire was supplemented with additional questions on vomiting, diarrhoea, constipation, colic and food allergies. With the exception of two allergic symptoms (wheeze and dry cough), mothers were asked to specify the cause of other allergic symptoms if known (appendix 6.1, section 2). Mothers were also asked whether they had consulted with their GP about any of these symptoms and where relevant, to specify the recommendation(s).

In addition, a list of allergenic foods as a possible cause of allergic symptoms (including wheat, egg,
milk, fish, nuts and sesame) was added to the questionnaire. This list was developed by the Enquiring About Tolerance (EAT) study team, to assess the feasibility of early introduction of allergenic foods in breast-fed infants on prevalence of food allergy (Perkin et al., 2015).

6.4. Methods

6.4.1. Study design and setting
Please see Chapter 5, section 5.4.1.

6.4.2. Study sample and recruitment
Please see Chapter 5, section 5.4.2.

6.4.3. Ethical considerations
Please see Chapter 5, section 5.4.3. An independent permission was also sought from the Allergy team in King’s College London, St Thomas’ hospital, in order to include the list of highly allergenic foods in the 6 months follow-up questionnaire.

6.4.4. Administration of questionnaire
As outlined in Chapter 5, section 5.4.4 midwives completed the wave 0 questionnaire (appendix 5.6) that also included family history of allergy asking whether either a parent of the infant or any sibling had ever had symptoms of asthma, hay fever, rash, wheeze, runny nose and food allergies.

The 6 month follow-up questionnaire for this PhD project was self-administered and women were provided with contact details of the research team to clarify any queries. The questionnaire and a prepaid envelope was posted to mothers when their babies were six months of age, and if they had not responded within three weeks, they received a phone reminder.

6.4.5. Questionnaire coding
Where possible, the questionnaire was coded and scored according to published standards. To ensure consistency in the coding of the questionnaire, a coding logbook was maintained. Data from individual questionnaires was used if at least 75% of the questionnaire had been completed.

6.4.5.1. Food Frequency Questionnaire (FFQ)
Consumption of the food items in the FFQ questionnaire was calculated per week, being consistent with the frequency options in the original questionnaire. To reflect consumption per week, a numeric value was assigned to each frequency category, ranging from 0 to 14 (never=0, 1-3 times per month=0.5, once
per week=1, twice per week=2, 3 times per week=3, 4 times per week=4, 5 times per week=5, 6 times per week=6, 7 times per week=7 and more than once a day=14). The frequencies of each contributing food item in the food groups were added to calculate the weekly frequency consumption of each food group (ready-made baby foods, starchy foods, vegetables, fruit, yogurt and fromage frais and meat/fish). The weekly consumption of drinks and water were also calculated as explained above. The weekly consumption of food groups and drinks were treated as continuous variables; therefore, higher frequency intakes of these equals a higher score. In addition, the scores of all food groups, excluding drinks, were added together to obtain a total score for all foods consumed where the higher score indicates higher consumption.

Missing replies to food items were replaced in the following way: if the baby had not been weaned (reply to “when did you first introduce solids into baby’s diet?” was answered “no, n/a”) and the replies to food items (allergenic and other food items) and drinks were left blank, it was decided to give a score of 0 to all the food items and drinks. If babies were weaned and there were missing replies to single food items, these were replaced by 0. Three mothers had started weaning and missed the replies to the list of allergenic foods and these were not replaced. The food items included in each food component and number of missing values are presented in appendix 6.2.

6.4.6. Data analysis
The Statistical Package for Social Sciences (SPSS) software (IBM, version 23) was used to analyse the data. Data sets were checked for outliers. Descriptive statistics were used to describe the data and all continuous variables were tested for normality using histograms. The mean and standard deviation (SD) were presented for normally distributed data, and otherwise median and range reported. Categorical variables were expressed as frequency and percentage. The χ² test was used for categorical variables. To assess the effect of multiple factors on the main outcome measures, multiple logistics regression analyses were conducted. The significance level for all analyses was defined at 0.05 level.

6.5. Results
Of 134 pregnant women who received the 6 months follow-up questionnaire, 80 responded (59.70%). Mean age of babies was 6.82 months (SD=0.89) and mothers had completed the questionnaires by themselves (100%).
6.5.1. Description of sample and by developing allergies in children

Detailed demographic characteristics of participants who responded to the 6 month follow-up questionnaire are shown in Table 5.2 (Chapter 5, section 5.5.1). Specific allergic symptoms and any allergies in children at 6 months of age is shown in Table 6.1. Wheezing was the most frequent allergic symptom reported (25%) and vomiting, constipation and colic were the most common food-related symptoms reported. Any allergies in children were defined, as whether they had experienced symptoms of “wheezing and dry cough” or when food was the cause for another allergic symptom (rash, vomiting, diarrhoea, constipation, colic and other food-related problems). In total, 42 children (52.7%) reported any symptoms of allergies. Also, ten babies (12.5%) had developed itchy rash and 4 were reported to be caused by food.

Twenty four mothers (30%) had consulted their GP or paediatrician about the allergic symptoms reported and recommendations were: referral to paediatrician (n=3), dairy intolerance/avoiding cow’s milk/lactulose (n=4), and the rest were either prescribed medications such as Gaviscon, infacol, ranitidine or did not require any further treatment.

Table 6.1. Allergic symptoms in children

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Yes</th>
<th>No</th>
<th>Don’t know</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheezing/whistling (n=80)</td>
<td>20</td>
<td>59</td>
<td>1</td>
</tr>
<tr>
<td>Dry cough at night (n=80)</td>
<td>8</td>
<td>71</td>
<td>1</td>
</tr>
<tr>
<td>Itchy rash (n=80)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Identified cause of rash</td>
<td>10</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>Rash caused by food</td>
<td>8</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Foods caused the rash</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Cow’s milk allergy</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vomiting (&gt;1tbsp) (n=80)</td>
<td>35</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>Identified cause of vomiting</td>
<td>29</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Vomiting caused by food</td>
<td>12</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Foods caused vomiting</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cow’s milk allergy (formula)</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orange</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dairy +soya</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reflux</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Too much milk</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weaning</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not specified</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhoea (n=80)</td>
<td>24</td>
<td>55</td>
<td>1</td>
</tr>
<tr>
<td>Identified cause of diarrhoea</td>
<td>17</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Diarrhoea caused by food</td>
<td>7</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Foods caused diarrhoea</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baby rice</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cow’s milk allergy</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk, yoghurt, cheese</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mum had pickled onion</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not sure what food</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Event</td>
<td>Yes N (%)</td>
<td>No N (%)</td>
<td>Don’t know N (%)</td>
</tr>
<tr>
<td>-------------------------------------------</td>
<td>-----------</td>
<td>----------</td>
<td>-----------------</td>
</tr>
<tr>
<td>Constipation (n=80)</td>
<td>26 (32.5)</td>
<td>52 (65.0)</td>
<td>2 (2.5)</td>
</tr>
<tr>
<td>Identified cause of constipation</td>
<td>18 (69.2)</td>
<td>6 (23.1)</td>
<td>2 (7.7)</td>
</tr>
<tr>
<td>Constipation caused by food</td>
<td>10 (55.6)</td>
<td>8 (44.4)</td>
<td>-</td>
</tr>
<tr>
<td>Foods caused constipation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carrots</td>
<td>1 (0.7)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Egg</td>
<td>1 (0.7)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gaviscon</td>
<td>1 (0.7)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Introduction of solids/weaning</td>
<td>2 (1.5)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Formula</td>
<td>2 (1.5)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Stay down milk/Gaviscon</td>
<td>1 (0.7)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Not specified</td>
<td>2 (1.5)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Colic/tummy ache</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Identified cause of colic</td>
<td>38 (47.5)</td>
<td>10 (26.3)</td>
<td>5 (13.2)</td>
</tr>
<tr>
<td>Colic caused by food</td>
<td>23 (60.5)</td>
<td>13 (56.5)</td>
<td>-</td>
</tr>
<tr>
<td>Foods caused colic</td>
<td>10 (43.5)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cow’s milk allergy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Curry (spicy)</td>
<td>4 (3.0)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dairy + soya</td>
<td>1 (0.7)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pickled onions (eaten by mum)</td>
<td>1 (0.7)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Did not specify</td>
<td>1 (0.7)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Other food related problems</td>
<td>8 (10.0)</td>
<td>71 (88.7)</td>
<td>1 (1.5)</td>
</tr>
<tr>
<td>Food-related problem</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Facial-rash</td>
<td>1 (0.7)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Possible acid reflux + stridor</td>
<td>1 (0.7)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Rash/vomit/poo/wind/sleep apnea/blistered lip</td>
<td>1 (0.7)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Reactions to dairy, soya, egg, nut, gluten</td>
<td>1 (0.7)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Reflux</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rash, but not itchy</td>
<td>1 (0.7)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sickness, diarrhoea, acid reflux</td>
<td>1 (0.7)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tummy ache + wind</td>
<td>1 (0.7)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cause of food-related problem</td>
<td>1 (0.7)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cow’s milk allergy</td>
<td>4 (3.0)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Garlic + onions</td>
<td>1 (0.7)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Soya milk in food</td>
<td>1 (0.7)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Not known/unidentified</td>
<td>2 (1.5)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Developing any allergic symptoms</td>
<td>42 (52.5)</td>
<td>38 (47.5)</td>
<td>-</td>
</tr>
</tbody>
</table>

### 6.5.2. Family history of allergies in the sample studied

The prevalence of family history of allergies in the overall sample, broken down by child allergy status at 6 months, is shown in Table 6.2. There were no associations between those with a maternal family history of asthma (p=0.48) or other allergies (p=0.73) with developing allergic symptoms at 6 months.
Table 6.2. Family history of allergies

<table>
<thead>
<tr>
<th>Family history</th>
<th>Responded to 6months questionnaire (n=80) N (%)</th>
<th>Allergic children (n=42) N (%)</th>
<th>Non-allergic children (n=38) N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Asthma</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother (n=79)*</td>
<td>15 (19.0)</td>
<td>9 (22.0)</td>
<td>6 (15.8)</td>
</tr>
<tr>
<td>Any* (n=79)</td>
<td>24 (30.4)</td>
<td>14 (34.1)</td>
<td>10 (26.3)</td>
</tr>
<tr>
<td><strong>Hay fever</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother (n=80)</td>
<td>23 (28.7)</td>
<td>12 (28.6)</td>
<td>11 (28.9)</td>
</tr>
<tr>
<td>Any (n=79)</td>
<td>41 (51.9)</td>
<td>21 (51.2)</td>
<td>20 (52.6)</td>
</tr>
<tr>
<td><strong>Rash</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother (n=80)</td>
<td>12 (15.0)</td>
<td>7 (16.7)</td>
<td>5 (13.2)</td>
</tr>
<tr>
<td>Any (n=79)</td>
<td>23 (29.1)</td>
<td>12 (29.3)</td>
<td>11 (28.9)</td>
</tr>
<tr>
<td><strong>Wheeze</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother (n=80)</td>
<td>18 (22.5)</td>
<td>12 (28.6)</td>
<td>6 (15.8)</td>
</tr>
<tr>
<td>Any (n=80)</td>
<td>30 (37.5)</td>
<td>19 (45.2)</td>
<td>11 (28.9)</td>
</tr>
<tr>
<td><strong>Runny nose</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother (n=80)</td>
<td>19 (23.8)</td>
<td>10 (23.8)</td>
<td>9 (23.7)</td>
</tr>
<tr>
<td>Any (n=80)</td>
<td>39 (48.8)</td>
<td>20 (47.6)</td>
<td>19 (50)</td>
</tr>
<tr>
<td><strong>Food allergy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother (n=80)</td>
<td>10 (12.5)</td>
<td>5 (11.9)</td>
<td>5 (13.2)</td>
</tr>
<tr>
<td>Any (n=80)</td>
<td>19 (23.8)</td>
<td>12 (28.6)</td>
<td>7 (18.4)</td>
</tr>
<tr>
<td>Any allergies</td>
<td>(n=80)</td>
<td>64 (80.0)</td>
<td>33 (78.6)</td>
</tr>
</tbody>
</table>

1. Difference between allergic and non-allergic children not significant using $\chi^2$ test
2. Family history of asthma/hay fever/rash/wheeze/runny nose/food allergy either in mother, father or sibling(s)

6.5.3. Maternal diet during pregnancy (AHEI score) and allergies in children

The measurement and calculation of AHEI score in pregnancy is outlined in Chapter 5, section 5.4.5.1. The association between maternal AHEI score and the development of allergies in children was assessed using an independent student $t$-test, where the mean of maternal AHEI score in pregnancy was not significantly different between allergic and non-allergic children (33.35 vs. 34.39, $p=0.72$).

6.5.4. Infant milk feeding type(s)

Details of type of milk infants consumed and age of weaning is shown in Table 6.3. At the time of data collection, 37.5% of infants were exclusively breast-fed whilst 52.5% were formula-fed. Mean age of solid food introduction was 22.3 weeks and some foods were avoided by ten infants (15.2%) due to reported allergies. Foods avoided were: any dairy and soya (N=1), cow’s milk (N=1), cow’s milk/yoghurt/butter (N=1), dairy/soya/nut/egg/gluten/wheat (N=1), egg and nuts until 12 months (N=1), nuts (N=2), orange (N=1), soy and milk (N=1) and one did not specify. No significant differences were found between the types of milk that infants consumed (breast vs. formula vs. mix of both), timing of introduction of solids and other variables included in Table 6.1.
<table>
<thead>
<tr>
<th>Baby feeding type</th>
<th>All (n=80) No. (%)</th>
<th>Allergic children (n=42) No (%)</th>
<th>Non-allergic children (n=38) No. (%)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exclusively breast milk</td>
<td>30 (37.5)</td>
<td>16 (38.1)</td>
<td>14 (36.8)</td>
<td>0.98</td>
</tr>
<tr>
<td>Exclusively formula</td>
<td>42 (52.5)</td>
<td>22 (52.4)</td>
<td>20 (52.6)</td>
<td></td>
</tr>
<tr>
<td>Mix of breast and formula</td>
<td>8 (10.0)</td>
<td>4 (9.5)</td>
<td>4 (10.5)</td>
<td></td>
</tr>
<tr>
<td>Solid food introduction (weeks)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;4months</td>
<td>2 (2.5)</td>
<td>1 (2.4)</td>
<td>1 (2.6)</td>
<td>0.95</td>
</tr>
<tr>
<td>&gt;6months</td>
<td>43 (53.8)</td>
<td>24 (57.1)</td>
<td>19 (50.0)</td>
<td></td>
</tr>
<tr>
<td>Not specified</td>
<td>20 (25.0)</td>
<td>9 (21.4)</td>
<td>11 (28.9)</td>
<td></td>
</tr>
<tr>
<td>Not started</td>
<td>4 (5.0)</td>
<td>2 (4.8)</td>
<td>2 (5.3)</td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>22.30 (3.53)</td>
<td>21.47 (3.55)</td>
<td>23.20 (3.33)</td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>23.0 (11-30)</td>
<td>21 (12-28)</td>
<td>24 (11-30)</td>
<td></td>
</tr>
<tr>
<td>Avoiding foods due to allergy</td>
<td></td>
<td></td>
<td></td>
<td>0.08</td>
</tr>
<tr>
<td>Yes</td>
<td>10 (12.5)</td>
<td>8 (19.0)</td>
<td>2 (5.3)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>56 (70.0)</td>
<td>27 (64.3)</td>
<td>29 (76.3)</td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>14 (17.5)</td>
<td>7 (16.7)</td>
<td>7 (18.4)</td>
<td></td>
</tr>
<tr>
<td>Type of weaning food</td>
<td></td>
<td></td>
<td></td>
<td>1.00</td>
</tr>
<tr>
<td>Home made</td>
<td>38 (47.5)</td>
<td>20 (47.6)</td>
<td>18 (47.4)</td>
<td></td>
</tr>
<tr>
<td>Shop bought</td>
<td>5 (6.3)</td>
<td>3 (7.1)</td>
<td>2 (5.3)</td>
<td></td>
</tr>
<tr>
<td>Mixture of both</td>
<td>23 (28.7)</td>
<td>12 (28.6)</td>
<td>11 (28.9)</td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>14 (17.5)</td>
<td>7 (16.7)</td>
<td>7 (18.4)</td>
<td></td>
</tr>
<tr>
<td>Dietary supplements</td>
<td></td>
<td></td>
<td></td>
<td>0.48</td>
</tr>
<tr>
<td>Yes</td>
<td>9 (11.20)</td>
<td>6 (14.3)</td>
<td>3 (7.9)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>56 (70.0)</td>
<td>28 (66.7)</td>
<td>28 (73.7)</td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>15 (18.8)</td>
<td>8 (19.0)</td>
<td>7 (18.4)</td>
<td></td>
</tr>
<tr>
<td>Attention paid to healthy-eating of baby</td>
<td></td>
<td></td>
<td></td>
<td>0.49</td>
</tr>
<tr>
<td>Very little</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>Somewhat</td>
<td>10 (12.5)</td>
<td>4 (9.5)</td>
<td>6 (15.8)</td>
<td></td>
</tr>
<tr>
<td>A great deal</td>
<td>56 (70.0)</td>
<td>31 (73.8)</td>
<td>25 (65.8)</td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>14 (17.5)</td>
<td>7 (16.7)</td>
<td>7 (18.4)</td>
<td></td>
</tr>
</tbody>
</table>

*Fishers’ exact test

6.5.5. Introduction of allergenic foods

The allergenic foods introduced to the diet of infants are shown in Table 6.4. With the exception of one infant who was exposed to cow’s milk earlier than 3 months of age, none of the other allergenic foods were introduced at this age. Overall, the most commonly allergenic foods introduced between 3 and 6 months and after 6 months were wheat (37.6% and 27.3%) and milk (22.1% and 31.2%). The least commonly allergenic foods introduced between 3 and 6 months and after 6 months were nuts (1.3% and 9.1%) and sesame (3.9% and 5.2%). Differences between allergic and non-allergic children for the intake of each allergenic food were not significant (Table 6.4).
### Table 6.4. Introduction of allergenic foods to baby diet (n=77)

<table>
<thead>
<tr>
<th></th>
<th>All (n=77) No. (%)</th>
<th>Allergic children (n=41) No. (%)</th>
<th>Non-allergic children (n=36) No. (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Wheat</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>27 (35.1)</td>
<td>18 (43.9)</td>
<td>9 (25.0)</td>
<td>0.06***</td>
</tr>
<tr>
<td>&lt;3 months</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>3-6 months</td>
<td>29 (37.6)</td>
<td>16 (39.0)</td>
<td>13 (36.1)</td>
<td></td>
</tr>
<tr>
<td>&gt;6 months</td>
<td>21 (27.3)</td>
<td>7 (17.1)</td>
<td>14 (38.9)</td>
<td></td>
</tr>
<tr>
<td><strong>Egg</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>57 (74.0)</td>
<td>33 (80.5)</td>
<td>24 (66.7)</td>
<td>0.34</td>
</tr>
<tr>
<td>&lt;3 months</td>
<td>4 (5.2)</td>
<td>2 (4.9)</td>
<td>2 (5.6)</td>
<td></td>
</tr>
<tr>
<td>3-6 months</td>
<td>16 (20.8)</td>
<td>6 (14.6)</td>
<td>10 (27.7)</td>
<td></td>
</tr>
<tr>
<td>&gt;6 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Milk</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>35 (45.5)</td>
<td>21 (51.2)</td>
<td>14 (38.9)</td>
<td>0.36***</td>
</tr>
<tr>
<td>&lt;3 months</td>
<td>1 (1.3)</td>
<td>1 (2.4)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>3-6 months</td>
<td>17 (22.1)</td>
<td>9 (22.0)</td>
<td>8 (22.2)</td>
<td></td>
</tr>
<tr>
<td>&gt;6 months</td>
<td>24 (31.2)</td>
<td>10 (24.4)</td>
<td>14 (38.9)</td>
<td></td>
</tr>
<tr>
<td><strong>Fish</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>56 (72.7)</td>
<td>31 (75.6)</td>
<td>25 (69.4)</td>
<td>0.08***</td>
</tr>
<tr>
<td>&lt;3 months</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>3-6 months</td>
<td>6 (7.8)</td>
<td>5 (12.2)</td>
<td>1 (2.8)</td>
<td></td>
</tr>
<tr>
<td>&gt;6 months</td>
<td>15 (19.5)</td>
<td>5 (12.2)</td>
<td>10 (27.8)</td>
<td></td>
</tr>
<tr>
<td><strong>Nuts</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>69 (89.6)</td>
<td>38 (92.7)</td>
<td>31 (86.1)</td>
<td>0.38***</td>
</tr>
<tr>
<td>&lt;3 months</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>3-6 months</td>
<td>1 (1.3)</td>
<td>-</td>
<td>1 (2.8)</td>
<td></td>
</tr>
<tr>
<td>&gt;6 months</td>
<td>7 (9.1)</td>
<td>3 (7.3)</td>
<td>4 (11.1)</td>
<td></td>
</tr>
<tr>
<td><strong>Sesame</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>70 (90.9)</td>
<td>38 (92.7)</td>
<td>32 (88.9)</td>
<td>0.45***</td>
</tr>
<tr>
<td>&lt;3 months</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>3-6 months</td>
<td>3 (3.9)</td>
<td>2 (4.9)</td>
<td>1 (2.8)</td>
<td></td>
</tr>
<tr>
<td>&gt;6 months</td>
<td>4 (5.2)</td>
<td>1 (2.4)</td>
<td>3 (8.3)</td>
<td></td>
</tr>
</tbody>
</table>

*There were three missing replies to these questions. χ² test Fisher’s exact test

### 6.5.6. Infant food frequency questionnaire

Median frequency consumption of different food groups is shown in Table 6.5. Criteria for grouping and scoring the food groups are described in section 6.4.5.1. The median consumption of ready-made baby foods, starchy carbohydrates and yogurt/fromage frais were lower in allergic children, while the consumption of vegetables and fruit were very similar. Introduction of meat/fish and non-water drinks were very low in either group and water had a similar pattern. In total, there were no significant differences in consumption of the food groups between allergic and non-allergic children (Table 6.6). When considering all foods, the allergic children scored lower than non-allergic children (19.75 vs. 22.75) meaning the intake of foods was less varied in allergic children; however the difference was not significant (p=0.81).
Table 6.5. Median frequency consumption of food groups and drinks per week

<table>
<thead>
<tr>
<th></th>
<th>All (n=80) Median (range)</th>
<th>Allergic children (n=42) Median (range)</th>
<th>Non-allergic children (n=38) Median (range)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ready-made baby foods</td>
<td>4.75 (0-33)</td>
<td>3.25 (0-29.5)</td>
<td>5.5 (0-33)</td>
<td>0.81</td>
</tr>
<tr>
<td>Starchy carbohydrates</td>
<td>2.0 (0-23.5)</td>
<td>1.75 (0-23)</td>
<td>2.25 (0-23)</td>
<td>0.80</td>
</tr>
<tr>
<td>Meat and fish</td>
<td>0.0 (0-28)</td>
<td>0.0 (0-8)</td>
<td>0.0 (0-28)</td>
<td>0.46</td>
</tr>
<tr>
<td>Vegetables</td>
<td>5.0 (0-18)</td>
<td>5.0 (0-16)</td>
<td>5 (0-18)</td>
<td>0.69</td>
</tr>
<tr>
<td>Fruit</td>
<td>4.0 (0-19)</td>
<td>3.75 (0-19)</td>
<td>4.0 (0-18)</td>
<td>0.72</td>
</tr>
<tr>
<td>Yogurt, fromage frais</td>
<td>0.0 (0-7)</td>
<td>0.0 (0-7)</td>
<td>0.5 (0-7)</td>
<td>0.93</td>
</tr>
<tr>
<td>Non water drinks</td>
<td>0.0 (0-14)</td>
<td>0.0 (0-14)</td>
<td>0.0 (0-14)</td>
<td>0.33</td>
</tr>
<tr>
<td>Water</td>
<td>7.0 (0-14)</td>
<td>7.0 (0-14)</td>
<td>7.0 (0-14)</td>
<td>0.73</td>
</tr>
<tr>
<td>All foods **</td>
<td>21.25 (0-95)</td>
<td>19.75 (0-75)</td>
<td>22.75 (0-95)</td>
<td>0.81</td>
</tr>
</tbody>
</table>

χ² test
Not including non-water drinks and water

6.5.7. Predictive effects of defined risk factors on developing allergies in children

Logistic regression analysis was undertaken to investigate the impact of all defined risk factors on developing allergies at 6 months of age (Table 6.6). The introduction of solids was introduced as a binary factor into the model where the “less than 4 months” (2 babies) and “between 4-6 months” categories were grouped together and also, the “not started weaning” (11 babies) was combined with “more than 6 months” category. This was done to yield plausible numbers within groups for statistical purposes. Of the factors included in the model, only introduction of wheat at 3-6 months was found to be associated with allergies in children with a protective effect (β=-2.07, p=0.04), although its introduction after 6 months showed a borderline association (β=-2.47, p=0.06).
### Table 6.6. Logistic regression model for predictors of allergies in babies

<table>
<thead>
<tr>
<th></th>
<th>β (SE)</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Main model</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family history of allergies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1 (ref)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>-0.33 (0.81)</td>
<td>0.14 - 3.52</td>
<td>0.68</td>
</tr>
<tr>
<td>AHEI-P score</td>
<td>0.005 (0.03)</td>
<td>0.94 - 1.07</td>
<td>0.87</td>
</tr>
<tr>
<td>Infant milk-fed type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exclusively breast-fed</td>
<td>Ref (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exclusively formula-fed</td>
<td>-0.42 (0.76)</td>
<td>0.14 - 2.90</td>
<td>0.57</td>
</tr>
<tr>
<td>Partially breast-fed</td>
<td>-0.03 (1.15)</td>
<td>0.10 - 9.35</td>
<td>0.97</td>
</tr>
<tr>
<td>Wheat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>Ref (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-6 months</td>
<td>-2.07 (1.00)</td>
<td>0.14 - 0.90</td>
<td>0.04</td>
</tr>
<tr>
<td>&gt;6months</td>
<td>-2.47 (1.33)</td>
<td>0.006 - 1.16</td>
<td>0.06</td>
</tr>
<tr>
<td>Egg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>Ref (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-6 months</td>
<td>-0.56 (1.79)</td>
<td>0.01 - 19.10</td>
<td>0.75</td>
</tr>
<tr>
<td>&gt;6months</td>
<td>-1.58 (1.39)</td>
<td>0.01 - 3.17</td>
<td>0.25</td>
</tr>
<tr>
<td>Milk</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>Ref (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-6 months</td>
<td>0.43 (0.99)</td>
<td>0.21 - 10.90</td>
<td>0.66</td>
</tr>
<tr>
<td>&gt;6months</td>
<td>0.26 (1.19)</td>
<td>0.12 - 13.38</td>
<td>0.82</td>
</tr>
<tr>
<td>Fish</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>Ref (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-6 months</td>
<td>0.04 (1.78)</td>
<td>0.03 - 34.31</td>
<td>0.97</td>
</tr>
<tr>
<td>&gt;6months</td>
<td>0.71 (1.30)</td>
<td>0.15 - 26.21</td>
<td>0.58</td>
</tr>
<tr>
<td>Nuts</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>Ref (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-6 months</td>
<td>1.34 (1.53)</td>
<td>0.19 - 77.12</td>
<td>0.37</td>
</tr>
<tr>
<td>&gt;6months</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sesame</td>
<td></td>
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<tr>
<td>Never</td>
<td>Ref (1)</td>
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<tr>
<td>3-6 months</td>
<td>0.16 (1.63)</td>
<td>0.04 - 29.18</td>
<td>0.92</td>
</tr>
<tr>
<td>&gt;6months</td>
<td>-2.01 (1.92)</td>
<td>0.003 - 5.81</td>
<td>0.29</td>
</tr>
<tr>
<td>Other foods (total score)</td>
<td>0.02 (0.02)</td>
<td>0.98 - 1.08</td>
<td>0.24</td>
</tr>
<tr>
<td>Solids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;6months</td>
<td>Ref (1)</td>
<td></td>
<td></td>
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<tr>
<td>4-6months</td>
<td>1.01 (0.89)</td>
<td>0.06 - 2.10</td>
<td>0.26</td>
</tr>
</tbody>
</table>

*Only one baby was introduced and the analysis not reported

**Including ready-made baby foods, starchy carbohydrates, meat & fish, vegetable, fruits, yogurt

The family history of maternal asthma (n=67), on its own and also together with the family history of all allergies, was included in the model and the results did not change.

### 6.6. Discussion

This cohort study set out to assess the effects of family history of allergies, quality of maternal diet during pregnancy and feeding practices in infants on the development of allergies at 6 months of age. There were no significant associations between the defined individual primary risk factors and allergies in children at 6 months. Assessing the influence of all the risk factors in a logistic regression model showed that
consumption of wheat between 3-6 months had a protective effect on the development of allergies at 6 months of age.

6.6.1. Allergic symptoms reported in children
In total, 52.7% of infants in this study reported any symptoms of allergies, and wheezing was the most reported allergic symptom followed by the frequently food-related symptoms of vomiting, constipation and colic. It is important to add that the allergic symptoms were reported symptoms by parents and no clinical tests such as SPT were used to assess sensitisation. As outlined in Chapter 2, allergies frequently begin early on in infancy and infants are prone to developing atopic dermatitis, food allergies and recurrent wheezing whereas asthma and allergic rhinitis usually occur later in childhood (Bieber, et al., 2013). In this sample of infants, cow’s milk allergy appeared to be the most common allergy, as reported by mothers and their GP consultation (Table 6.1). Studies indicate that manifestation of allergies to cow’s milk protein (CMP) is most common in the first few months when the infant is exposed to cow’s milk via breast milk, infant formula or solid foods; however most infants develop tolerance by late childhood (Allen & Koplin, 2012). The symptoms of allergy in this study were taken from a standardised questionnaire (Asher, et al., 1995) and it was supplemented with additional allergic symptoms. There is a possibility that reporting of allergic symptoms has been influenced by a different understanding of the questions among individuals, for example, the interpretation for the definition of wheeze or colic may vary between people.

There were no links between either maternal family history of asthma or any other allergies with allergic symptoms in the studied sample, and it is possible that the study was underpowered to detect any associations. As described in Chapter 2 (section 2.5.1) family history of allergy plays an important role in developing allergies in child particularly for maternal asthma ((Böhme, et al., 2003; Kjellman & Johansson, 1976; Koppelman, et al., 1999).

6.6.2. Maternal diet during pregnancy
The present study did not find an association between mean score of AHEI-P, as a proxy measure for quality of maternal diet during pregnancy, and likelihood of developing allergies in children at 6 months of age. This could be because the study was not sufficiently powered; however this result is consistent with two studies conducted in the US (Lange et al., 2010) and the UK (Moonesinghe et al., 2016), that found no association between maternal HEI and childhood asthma and other allergies. These studies had a follow-up duration of 3 and 10 years respectively, and the UK study used an allergy-focused HEI diet.
As described in Chapter 5, diet could be assessed using either an a priori (based on previous knowledge) or posteriori approach (dietary patterns) and the current evidence in pregnant women heavily relies on knowledge from eating style patterns among different populations. Literature around maternal diet during pregnancy and childhood allergies is already outlined in Chapter 2 (section 2.5.2) and in summary, evidence from a systematic review and meta-analysis of cohort studies (Beckhaus et al., 2015) only showed protective effects for maternal consumption of certain nutrients (Vitamins D, E and zinc) in pregnancy on childhood wheeze and a few studies contributed to their meta-analysis. A recent narrative review assessing the evidence on adherence to Mediterranean style diet during pregnancy showed the eating pattern is beneficial for childhood asthma/wheeze during the first year of life but no promising effects for other allergic outcomes were reported (Castro-Rodriguez & Garcia-Marcos, 2017). A further study from Singapore (Loo et al., 2017) also showed that, of the emergent dietary patterns, only the seafood and noodles style showed a beneficial effect on allergen sensitisation at 18 and 36 months of age in an adjusted analysis. To conclude, there is a paucity of evidence from studies assessing HEI in pregnancy and childhood allergies and nevertheless, current results from both these studies and dietary patterns in pregnant women seem to be inconclusive due to large heterogeneities between studies in terms of the definition and measurement of diet. This would suggest there is a need for well-defined studies to address maternal diet as a risk factor for childhood allergies.

### 6.6.3. Infant milk feeding practices

At 6 months of age, the rate of exclusive breast and formula feeding were 37.5% and 52.5% respectively, indicating that breast-feeding in this sample was slightly above the average national figure where 34% of infants are being breast-fed at 6 months old (McAndrew et al., 2010). The type of milk that infants consumed was not associated with allergies in children at 6 months. The role of breast-feeding on childhood allergies has been the subject of a number of studies and the relevant literature is discussed in Chapter 2 (section 2.5.3). In brief, a systematic review of studies with varied designs showed that duration of breast-feeding (more vs. less) decreased the risk of asthma at 5-18 years and also allergic rhinitis by ≤5 years and eczema by ≤2 years of age; however, high levels of heterogeneity were found in the conducted meta-analyses (Lodge et al., 2015). These results however, do not directly compare to the current study since this study only followed-up children at 6 months old. Also because the diagnosis of allergies in very early childhood is difficult, occurrence of allergies was defined by symptoms for common allergies caused by food; additionally, the study might be underpowered to identify these associations.
6.6.4. Timing of introduction of solids and allergenic foods

Nationally, 75% of babies are introduced to solids by five months old (McAndrew et al., 2010). In this study, most babies were weaned between 4-6 months age (53.8%) and statistically, there were no significant differences between the age of introduction of solids and developing allergies in children. Also, introduction of allergenic foods at different ages was not associated with subsequent development of allergies in children, although, in the logistic regression model, babies who were exposed to wheat between 3-6 months of age had lower prevalence of allergies (p=0.04). Prevention of food allergies by early introduction of common food allergens (peanut, cooked hen’s egg, cow’s milk, sesame, white fish and wheat) from 3 months of age in the EAT trial conducted in the UK failed to show a beneficial effect in the ITT analysis and further analysis of their data suggested that adherence and dose of these foods might play a role (Perkin et al., 2016). However, this RCT included exclusive breast-feeding babies and assessed the occurrence of food allergy by means of SPT and therefore, the results are not directly comparable with this observational study where interaction between the defined risk factors was assessed in a regression model.

The history and evidence around the introduction of allergenic foods into the infant diet and prevention of allergies from observational studies and RCTs is detailed in Chapter 2 (section 2.5.4). As shown in the review by Ierodiakonou and colleagues (2016) the current knowledge from observational studies on the topic is inconclusive, although the results from RCTs have incited some revisions only for introduction of peanuts in practice for early prevention of childhood allergies among professional bodies and allergists. A recent study from Singapore (Tham et al., 2017) reported that the prevalence of food allergy in a cohort of 1,152 children was low (between 0.1% to 1.8%) at 18 and 36 months of age in adjusted analysis, despite the late introduction (after 10 months of age) of allergenic foods into the infant diet. The findings from this large observational study highlight the fact that early introduction of allergenic foods might not be a practical approach in all populations and suggests that international/national guidelines for prevention of allergies need to be revised and adapted based on the ethnic/geographical differences and epidemiology of food allergies in countries.

6.6.5. Consumption of food groups in infants

Consumption of food groups between the allergic and non-allergic children did not significantly differ, although with the exception of vegetable and fruit groups, allergic children scored lower for the median consumption of most food groups. In addition, allergic children scored lower for overall food indicating that they had a less varied diet compared to the non-allergic group; however this was not statistically significant. Also, type of food in terms of home-made vs. shop bought or both was not significantly
different between allergic and non-allergic children, although 17.5% did not specify because they had not started on weaning and there were also missing data for this item. Of note, the current study provides data on the initial stages of weaning in infants where only 56.3% of mothers had started weaning by 6 months of age. In addition, diet at this very young age is not very diverse but is expected to be within a few months. Hence, the associations between variety of foods consumed and allergy status at this early age may not be representative of later ages. A study from the UK with a nested case-control design reported that the early infant diet pattern (in the first year of life) was not different between children with and without food allergies, whereas the ongoing diet pattern by 2 years of age was significantly different between the groups (Grimshaw et al., 2014), where non-allergic children ate a diet richer in fruit, vegetables and home-prepared foods (healthy diet). A large birth cohort from the UK also showed that a healthy dietary pattern was linked to lower risk of food hypersensitivity by 2 years of age in both IgE and non-IgE mediated phenotypes (Grimshaw et al., 2015). In this PhD study, patterns of intake for vegetables and fruit in allergic children were similar to that of non-allergic children, which could be because mothers perceive these foods as safe. However the results from this PhD study cannot be directly compared to the aforementioned studies where the diagnosis of food allergy was conducted by means of DBPCFC and children were followed-up by 2 years age. A larger study on this population with a longer follow-up duration could replicate the results.

An observational study from five European countries has furthermore reported that diversity of food in the first year of life, measured for 12 food/food categories, can have a protective effect on the development of asthma, food allergies and food sensitisation by 6 years of age (Roduit et al., 2014). The observed association persisted when children with food allergies were excluded from their analysis thus eliminating the reverse causality. Generally speaking, it can be hypothesised that it is the diet pattern that contributes towards the development of food allergies in children, or having a food allergy in infancy is the resulting factor for the diet pattern. Altogether, a causation effect cannot be inferred from either the discussed papers or current PhD study because of their prospective nature and yet, it appears that quality of diet early in life is an underlying factor for allergies in older age.

6.6.6. Strengths
Validated questionnaires designed for the target age group were used to collect data on symptoms of allergies and infant diet. The ISAAC questionnaire was supplemented with additional questions on gastrointestinal symptoms e.g. colic or diarrhea, to assist with identification of allergic symptoms at this very young age and whether they were caused by food(s), were deemed as allergic symptoms. Other strengths of the study are as previously outlined in Chapter 5 (section 5.6.5).
6.6.7. Limitations
Lack of use of clinical test(s) for the diagnosis of allergies in children is a key limitation for this cohort study. In addition, the short-term follow-up by 6 months age meant that direct comparison with other studies could not be made. Other limitations related to sample size and measurement of maternal diet during pregnancy are as previously explained in Chapter 5 (section 5.6.4).

6.6.8. Conclusion
The effect of maternal diet during pregnancy and feeding practices of babies early in life on the development of allergies at 6 months of age remains inconclusive and demands further investigation.
Chapter 7: General discussion and conclusion

7.1. Overview

The overall findings of this PhD study are brought together in this chapter. A recap of the rationale and principal aims for the research are presented followed by the main findings of the two studies conducted in this PhD and their implications. The methodological strengths and limitations are addressed and future research needs are discussed.

7.2. Rationale, hypothesis and aims of this programme of research

The main aim of this research was to examine the role of nutrition in pregnancy and early in life on the development of allergies and obesity in children and was conducted as two separate complementary studies. The first study aimed to synthesise the evidence of effectiveness of prenatal nutritional/dietary interventions on prevention of childhood allergies and obesity. The rationale for this was in response to an increasing trend of childhood allergies and obesity, avenues of research for prenatal nutritional interventions have been proposed as the first line for primary prevention of these conditions. Previous reviews have assessed the effect of some of the nutritional interventions during pregnancy, and mostly also included postnatal interventions and reported single allergic outcomes e.g. asthma (please see respective section 3.3.2.2) and obesity e.g. BMI (please see respective section 4.3.2.2). The evidence base for nutritional interventions that were conducted solely during pregnancy and which assessed the occurrence of overall allergic and obesity outcomes was extremely limited and not synthesised. Consequently, the first study of this PhD research programme intended to synthesise the most up to date evidence on the nutritional interventions administered only throughout pregnancy with a minimum follow-up of one month after birth, and incorporates a comprehensive range of allergic and obesity outcomes in children.

The second study of this research programme collected data prospectively in the Portsmouth birth cohort registry to investigate the associations between quality of maternal diet during pregnancy and early life feeding practices on weight/Z-score outcomes and allergies in children by 6 months of age. As discussed in detail in Chapter 2, because of large heterogeneities that exist between studies, there are a number of unanswered questions relating to the associations between maternal diet in pregnant women and feeding practices in early life, with the development of childhood allergies and obesity. In addition, quality of maternal diet using national/international guidelines is assessed in a few studies and most studies have reported dietary patterns which is data-driven using statistical methods; hence the subject is yet to be
assessed in further studies. Furthermore, the health status of the population in Portsmouth is below the national average and the results of this study, as the first survey conducted in this understudied population, could provide robust and reliable data for appropriate public health policies to promote the health and well-being of the residents, and also other regions with comparable population.

7.3. Main findings of the research

7.3.1. Nutritional interventions during pregnancy for prevention of childhood allergies

Findings of meta-analyses provided evidence that consumption of probiotics during pregnancy only had a protective effect on childhood eczema. A protective effect was also found for prenatal supplementation with fatty acids and the development of sensitisation in children. It should be noted that the high heterogeneity between the studies such as variability of the supplementation (probiotics, fatty acids), dosage and timing of interventions limits the confidence we have in the conclusiveness of these results. Therefore, the current evidence cannot be definitely translated into practice and further research via multi-centre, well-executed, harmonised RCTs are necessary. Also, given the immunomodulatory effects of both probiotics and fatty acids, their efficacy on reducing the development of other childhood allergies should be the subject for further research.

There was no evidence that food avoidance during pregnancy could protect against childhood allergies, indicating that the food avoidance theory favoured in the 1980s does not appear to hold true. Infancy is a plasticity period during which the development of the immune system occurs and in fact, early exposure to allergenic foods via maternal diet, particularly in the first trimester, could develop tolerance in the foetus but sensitising to allergens (Devereux et al., 2006; Julia, Macia, & Dombrowicz, 2015). These findings in line with the current guidelines highlight the fact that women need to follow a normal and balanced diet with no restrictions during pregnancy (Australasian Society of Clinical Immunology and Allergy, 2016; Boyce et al., 2011; Muraro et al., 2014).

The meta-analysis of studies that supplemented pregnant women with vitamins showed that prenatal consumption of Vitamin D could be associated with the development of childhood wheeze. The certainty of evidence is, however, low due to a paucity of studies and heterogeneities between these studies; nonetheless this novel finding could have valuable implications in practice for early prevention of childhood wheezing, which could have implications for the subsequent development of asthma given that this is one of the main risk factors for its development later in life. Further follow-ups of these studies are
also warranted to determine the longer-term effects of prenatal Vitamin D intervention. The anti-oxidative effects of vitamins on human immune function makes a strong theoretical case for their ability to prevent childhood allergies, but given the current paucity of evidence, it should be an important priority to assess the effectiveness of vitamins for prevention of allergies in larger well-designed trials.

7.3.2. Nutritional interventions during pregnancy for prevention of childhood obesity

No evidence was found that prenatal nutritional interventions of fatty acids and probiotics could have a protective effect on obesity outcomes in children. However this statement stems from a limited number of studies with substantial heterogeneity e.g. dosage/type of supplement and timing of intervention and furthermore, most of the studies were primarily conducted to measure outcomes other than obesity. Therefore, the evidence for the influential role of these supplements on childhood obesity is not yet definitive and demands further research. Combined use of these nutritional supplements with a balanced diet during pregnancy could also be considered in future studies.

A few studies investigated the effect of LG index diet and life-style change as dietary interventions in pregnancy on childhood obesity, and because the studies reported different outcomes it was not possible to conduct meta-analyses. These studies had a large loss to follow-up and their individual results within each dietary group were not promising for the reported obesity outcomes in infants. It is worth noting that women in the intervention groups, within both dietary classes, achieved modest improvements in the quality of their diet and/or level of physical activity. However, it appears that there is a low quality of evidence for the efficacy of these prenatal dietary interventions on infant outcomes, which calls for further large-scale trials. Effective approaches also need to be defined in order to maintain compliance with the intervention and minimise the rate of attrition. As previously discussed in Chapter 2, the in utero environment programmes the foetus from the very early days; hence, theoretically at least, timely maternal dietary interventions could be the potential solution for a favourable prenatal environment and childhood outcomes.

7.3.3. Findings of the cohort studies in relation to maternal diet during pregnancy

The quality of maternal diet in this sample of pregnant women was low, on average women scored 33.6 out of the maximum possible 70 for the AHEI-P score. A main distinction of the current study is that it measured the quality of maternal diet using a HEI score, which is based on the UK national guidelines for healthy eating in pregnancy. Relatively high frequency consumptions were also found for unhealthy items (sugar, processed meat and junk foods). These findings, as the first study in the city of Portsmouth, are concerning and highlight the need for public health policies to address the underlying factors for the
nutritional behaviours/choices within this understudied population. Other research worldwide has also shown that generally pregnant women do not meet the national nutrition recommendations and reported unfavourable intake for many nutrients (Blumfield, et al., 2012; Fowler & Evers, 2012; Malek, et al., 2015; Morton et al., 2014). Indeed, a number of factors such as pregnancy-related, demographic and environmental elements could affect the quality and quantity of diet in women at reproductive age (Doyle, et al., 2016; Malek, et al., 2015) and this needs to be addressed in further research within this population.

7.3.4. Findings of the cohort study in relation to the associations between the quality of maternal diet and weight/age outcomes in children

The weight/age Z-scores at birth and follow-ups were not associated with maternal AHEI-P score. However higher maternal sugar consumption was linked to lower weight/age Z-scores at birth and 6 months, although these effects disappeared when excluding the outliers on baby weight from the models. Negative associations were also found for formula-fed babies where higher maternal consumption of junk food and lower AHEI-P score were related with lower weight/age at 2 months. Overall, these findings support the importance of maternal diet during pregnancy since it could have implications for the risk of developing obesity later in life. As detailed earlier, different dietary patterns during pregnancy and also HEI scores could affect the risk of childhood obesity.

In this study, a small sample of pregnant women was included, and therefore further research with a larger sample is needed to replicate these findings. It must be highlighted that, in the current study, it was not possible to measure other indicators of obesity in infants e.g. BMI or body composition such as SFT, which limits the study findings to an extent. Moreover, the high prevalence of obesity globally has led to many women entering their pregnancy while obese. Obesity on one side could relate to the quality of maternal diet during pregnancy, while on the other side it could also affect infant growth and the later risk of obesity in the child. In this study, data on other maternal-related factors such as pre-pregnancy overweight/obesity and gestational weight gain were not available; this could have limited the regression models and thus the explanatory power of the model predicting weight/age outcomes in babies.

7.3.5. Findings of the cohort study in relation to the associations between quality of maternal diet and allergic outcomes in children

The likelihood of developing allergies in this sample of children was not linked to the quality of maternal diet during pregnancy. This supports the current evidence from the existing studies on the quality of maternal diet (assessed by HEI scores) and subsequent development of childhood allergies in offspring.
As previously discussed in Chapter 2, generally speaking, observational studies have reported mixed messages for the associations between the intakes of different nutrients or dietary patterns in pregnancy and the development of various phenotypes of allergy in children.

7.3.6. Findings of the cohort study in relation to infant feeding practices and weight/age outcomes

Partially breast-fed babies at 2 months of age weighed less for age at 2 and 6 months than exclusively breast and formula-fed babies and additionally, number of formula feeds was associated with higher weight at 2 months Z-score in exclusively formula-fed babies. The window of opportunity for programming the risk of life-long obesity extends into postnatal life. Breast-feeding in comparison to formula-feeding could influence a healthier growth trajectory characterised by lower weight gain and percentage body fat. Indeed, an overview of systematic reviews indicated that breast-feeding for any duration could reduce the risk of childhood overweight and obesity by 13% (Patro-Gołąb et al., 2016).

Rapid weight gain during the first year of life is also a strong predictor of childhood obesity (Druet et al., 2012) and in this context, the advantages of breast-feeding as a primary prevention for obesity need to be more significantly publicised. Timing of introduction of formula additionally has a significant role since weight gain during the first week of life in formula-fed infants poses a great risk of obesity later in life (Stettler et al., 2005). In this study sample, 20% of babies were bottle-fed on first day of birth and community interventions informing mothers of the very early risk factors for obesity in children could encourage healthier feeding practices in infancy.

7.3.7. Findings of the cohort study in relation to infant feeding practices and developing allergies at 6 months

Gastrointestinal conditions such as colic, diarrhoea and constipation are commonplace in infancy and are usually transient. In this study these were considered as symptoms of allergy when there appeared to be a link in their occurrence and the digestion of a certain food. In addition, occurrence of any allergies in children was based on parental-report of eight symptoms defined in the questionnaire, since diagnosis of allergy at this very young age is not definitive.

In this study, individual analyses for infant feeding practices (type of milk, timing of introduction of solids, introduction of allergenic foods and also other foods) did not find an association between these and the development of allergies. In the regression analysis, however, the protective effect for the early exposure to wheat between 3-6 months of age is very thought-provoking and adds weight to the argument
that early intake of allergenic food in infants could be safe, and may even be protective. This is certainly an area of current research focus, for example, the feasibility of early introduction of allergenic foods e.g. wheat and peanut has been investigated in a recent trial (Perkin et al., 2015).

The ideal time for introduction of solids into an infant’s diet is currently suggested after 6 months (World Health Organisation, 2003) and there is a controversy surrounding the risk of allergy if solids are offered after 6 months. Most infants are developmentally ready by 6 months of age in terms of being able to safely manage complementary feeding, and there is an argument that delayed weaning could increase the risk of allergies (Scientific Advisory Committee on Nutrition, 2017; Togias et al., 2017). In this study, 25% and 13.8% of babies were weaned after 6 months or not weaned yet respectively, suggesting the educational needs of parents in the city of Portsmouth with an emphasis that babies need to be exposed to a wide variety of age-appropriate nutritious foods in early life (Palmer & Prescott, 2017).

In addition, although babies in the current study were in the early stages of weaning, allergic children had a less varied diet than non-allergic children, scoring lower for the total intake of other foods although this was not significant. This has relevance for health professional practices in order to avoid unnecessary exclusion(s) from an infant’s diet since manipulation of dietary intake during infancy can have a profound effect on the later risk of allergies (Scientific Advisory Committee on Nutrition, 2017; Togias et al., 2017).

Generally speaking, the findings from the first study in this PhD provided some evidence for the importance of consumption of nutritional supplements in pregnant women and more specifically Vitamin D. In the second study, a small percentage of pregnant women reported that they consumed either any nutritional supplements (42.6%) or Vitamin D (33.8%) and detailed information for the consumption of probiotics, fatty acids was not assessed. The low consumption of Vitamin D suggests that the guidelines are not being well communicated to pregnant women and calls for health promotion initiatives.

7.4. Implications for future research

The first study of this research programme provided an up-to-date review of the evidence from studies of prenatal interventions for the prevention of allergic and obesity outcomes in the offspring and established some novel findings in relation to certain interventions i.e. probiotics, fatty acids and vitamins and the prevention of specific allergic outcomes. However, the heterogeneities observed between the conducted studies limit their findings in practice and calls for these to be addressed in further research. The key
potential for the maternal interventions during pregnancy is that they could modulate the gut microbiota and thus the immune system from very early in life, and, therefore, are a good primary prevention approach. The life-style interventions particularly need to target women in the preconception stage to regulate the maternal-related factors e.g. high BMI or diabetes that could directly affect the maturation of the foetal immune system during pregnancy. Given the multifactorial nature of allergies and obesity, future trials would ideally employ multifaceted schemes enabling them to control for other environmental factors such as quality of maternal diet to maximise their ability to assess the effectiveness of the interventions.

The second study of this research programme, as the first cohort study in the city of Portsmouth, investigated the relationship between quality of diet in pregnant women and weight outcomes and the development of allergies by 6 months of age. The results provided some evidence for the influential role of maternal diet on weight outcomes but not for allergies. However, longer-term follow-up of these babies would provide more reliable data on whether these effects will persist in later life.

The feeding practices of babies at 2 and 6 months of age also showed some associations with weight outcomes and allergies, which are not only novel findings as its first kind in the city of Portsmouth, but also could have implications in the other regions with a similar population. It is not possible, nonetheless, to predict whether these results will persist into later ages and further follow-up of these studies could assess to what extent the totality of diet at older age could affect the obesity and allergic outcomes in these children. It is worth noting that the longer-term follow-up is of more importance for assessing the allergic outcomes in these babies since there is no definitive diagnosis for allergies at a very early age, and their prevalence could also be affected by the diversity of foods digested as babies grow.

Furthermore, future research involving a larger sample of pregnant women and their babies would allow more detailed statistical analysis to be undertaken. For example it was not possible with the number of participants recruited in the current study, to differentiate the weight outcomes between exclusive and dominant breast-feeding practices, and, therefore, these were combined.

7.5. Strengths of the research

The first study of the research used an a priori published protocol that allowed complete coverage of the relevant literature. In addition, the systematic reviews only included trials that started the specific
nutritional/dietary intervention throughout pregnancy and a comprehensive range of childhood allergic and obesity outcomes were defined as the outcomes of interest in the reviews.

The main strength for the second study of research is that the cohort design allowed data to be collected prospectively and thus, minimising the recall bias. Dietary intake of pregnant women was also investigated for the total quality and a HEI score was calculated reflecting consumption of actual foods and food groups. The questionnaires used were all validated and age-appropriate for the follow-up assessments.

7.6. Limitations of the research

The limitations for the first study of research, synthesising the evidence from RCTs for the effectiveness of prenatal interventions for prevention of allergies and obesity outcomes in children, stems from the shortages in the studies conducted. These were mainly listed as differences in dosage/type/timing of intervention and issues in terms of sampling and duration of follow-up. There was also a deviation from the a priori protocol for this research in that the sub-group analyses (e.g. different strains of certain interventions, high vs. low risk participants) were not conducted in order to avoid a type two error or where these were already addressed in previous reviews. In addition, the standard for the conduct of systematic reviews is they are conducted within a team of researchers and for this PhD the student mainly conducted the reviews as a sole researcher. All the measures however, were carried out to assure the quality of the systematic reviews. For example, the main reviewer conducted cross-referencing and sought expert opinion to ensure that all the relevant studies were included in the reviews. Similarly, every effort was made to do the screening with great care and attention to detail, and any disagreement was discussed within the supervisory team. Also, any bias for the appraisal of studies and data extraction were minimised by crosschecking within the supervisory team. Furthermore, including the longest follow-up data from the included trials in the systematic reviews might have introduced heterogeneities into the meta-analyses since the length of follow-up between trials were varied however, this approach has provided insight for the longer term benefits of the interventions as opposed to the short-term effects. The findings of the systematic reviews, additionally, could have informed the observational study in this PhD. However, the time scale for completing this PhD research (particularly in light of the need to allow time for follow-up in the cohort study, and that the cohort study was part of a larger study with its own timescales) necessitated that these studies were undertaken concurrently.
For the second study of research, the key limitation was the small sample size, and also the short duration of follow-up. It was however an important opportunity to assess the short-term impact of the quality of maternal diet and infant feeding practices on growth and allergic outcomes and of course, further follow-up is an important tool to assess the impact as children grow. In addition, data on other growth parameters and obesity measures were not available and it was not feasible to collect data on maternal-related risk factors e.g. gestational weight gain.

The methodological issues concerning the collection of dietary data in pregnant women, using FFQ-P and also milk diary in infants, have previously been explained. However, these are inherent to nutritional assessment studies and this study attempted to choose appropriate dietary assessment methods for each target group. Using a logbook, possibility of any biases in the collected data and analysis were minimised. It is also acknowledged that nutritional intake of infants at 2 months of age were not validated against biomarkers and additionally, no clinical tests were used for the diagnosis of allergies at 6 months of age.

7.7. Concluding remarks

The original contribution of the first study of this research is that prenatal consumption of Vitamin D could protect against the development of wheeze in the offspring. The further follow-up of the included studies, however, could provide more robust data on whether the intervention could also prevent the development of childhood asthma.

The second studies conducted as cohort also provided evidence that unhealthy eating behaviours during pregnancy i.e. consumption of sugar and junk food, could have direct impact on weight outcomes in infants. Moreover, feeding practices early in life had an influence on the weight/Z-scores at 2 and 6 months of age where the partially breast-fed infants weighed lower. This has implications for public health policies to emphasise how any duration of breast-feeding could contribute to lower risk of obesity in life-long. Furthermore, the protective effect of early introduction of wheat between 3-6 months of age on the development of allergies at 6 months of age suggests that early introduction of allergenic foods could be considered beneficial.

The importance of the first 1,000 days in a baby’s life is highlighted in the introductory Chapter of this thesis where nutrition status in mother and infant could have an influential role on the proper maturation of an infant’s immune system. The findings from this PhD research have enhanced our understanding in
this field as to how early-life nutrition, as epigenetic environmental factor, can modify the risk of obesity and allergies in children.
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INTRODUCTION


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### Appendix 0.0. Form UPR16

**FORM UPR16**

**Research Ethics Review Checklist**

<table>
<thead>
<tr>
<th>Postgraduate Research Student (PGRS) Information</th>
<th>Student ID: 716645</th>
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<tbody>
<tr>
<td><strong>PGRS Name:</strong> Mariam Vahdaninia</td>
<td></td>
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<tr>
<td><strong>Department:</strong> SHSSW</td>
<td></td>
</tr>
<tr>
<td><strong>First Supervisor:</strong> Joint 1st supervisors: Professor Tara Dean, Dr Suzannah Helps</td>
<td></td>
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<tr>
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<table>
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<tr>
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If you are unsure about any of the following, please contact the local representative on your Faculty Ethics Committee for advice. Please note that it is your responsibility to follow the University’s Ethics Policy and any relevant University, academic or professional guidelines in the conduct of your study.

Although the Ethics Committee may have given your study a favourable opinion, the final responsibility for the ethical conduct of this work lies with the researcher(s).

**UKRIO Finished Research Checklist:**

(If you would like to know more about the checklist, please see your Faculty or Departmental Ethics Committee rep or see the online version of the full checklist at: [http://www.ukrio.org/what-we-do/code-of-practice-for-research/](http://www.ukrio.org/what-we-do/code-of-practice-for-research/))

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<tr>
<th>a) Have all of your research and findings been reported accurately, honestly and within a reasonable time frame?</th>
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<th>NO ☑️</th>
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<td>b) Have all contributions to knowledge been acknowledged?</td>
<td>YES ☑️</td>
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<td>c) Have you complied with all agreements relating to intellectual property, publication and authorship?</td>
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<tr>
<td>d) Has your research data been retained in a secure and accessible form and will it remain so for the required duration?</td>
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<tr>
<td>e) Does your research comply with all legal, ethical, and contractual requirements?</td>
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**Candidate Statement:**

I have considered the ethical dimensions of the above named research project, and have successfully obtained the necessary ethical approval(s)
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<td>If you have <em>not</em> submitted your work for ethical review, and/or you have answered ‘No’ to one or more of questions a) to e), please explain below why this is so:</td>
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</tr>
<tr>
<td>All the required ethical approvals for the Cohort study in this PhD research project have been obtained from the Berkshire NHS Research Ethics Committee and the relevant documents are presented as appendices in the final thesis file.</td>
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<tr>
<td>Signed (PGRS):</td>
<td>Date: 28.02.2019</td>
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Appendix 0.1. Article “Prenatal intake of vitamins and allergic outcomes in the offspring: a systematic review and meta-analysis”

**ARTICLE IN PRESS**

**Original Article**

**Prenatal Intake of Vitamins and Allergic Outcomes in the Offspring: A Systematic Review and Meta-Analysis**

Mariam Vahdaninia, BSc, MSc\(^b\), Heather Mackenzie, PhD\(^b\), Suzannah Helps, PhD\(^b\), and Taraneg Dean, PhD\(^a,b\)  
Portsmouth and Brighton, United Kingdom

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BACKGROUND: Allergic diseases have seen a rise worldwide, with children suffering the highest burden. Thus, early prevention of allergic diseases is a public health priority.  
OBJECTIVE: To synthesize the evidence from randomized controlled trials (RCTs) assessing the effect of vitamin interventions during pregnancy on developing allergic diseases in offspring.  
METHODS: We searched CENTRAL, MEDLINE, SCOPUS, World Health Organization’s International Clinical Trials Registration, E-theses, and Web of Science. Study quality was evaluated using Cochrane’s risk of bias tool. Included RCTs had a minimum of 1-month follow-up postpartum.  
RESULTS: A total of 5 RCTs met the inclusion criteria, including 2,456 children who used vitamins C + E (1 study), vitamin C (1 study), and vitamin D (3 studies) compared with placebo/control. Two studies were judged to have a high risk of bias for performance bias or a high rate of loss to follow-up. All were rated as low risk of bias for blinding of outcome assessment. We did not perform meta-analysis with vitamin C or vitamin C + E studies due to high heterogeneity between the 2 included studies. However, we did conduct a meta-analysis with trials on vitamin D (including 1,493 children) and the results showed an association between the prenatal intake of vitamin D and the risk of developing recurrent wheeze in offspring (relative risk (RR): 0.812; 95% CI: 0.67–0.98).  
CONCLUSIONS: The current evidence suggests that prenatal supplementation of vitamin D might have a beneficial effect on recurrent wheezing in children. Longer-term follow-up of these studies is needed to ascertain whether this observed effect is sustained. There is lack of evidence on the effect of other vitamins for the prevention of respiratory and/or allergic outcomes. © 2016 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2016;11:1-11)

**Key words:** Vitamins; Allergic outcomes; Asthma; Wheeze; Wheezing; Respiratory outcomes; Eczema; Offspring; Clinical trial; Intervention; Efficacy; Effectiveness; Systematic review; Meta-analysis

In the last 2 decades, allergic diseases have seen a rise worldwide, with children suffering the highest burden of the condition.  
Food allergies, eczema, and asthma are the most common allergic disorders in children. Because of the increasing burden of allergic diseases, they are a key focus for public health.  
The Developmental Origins of Health and Disease theory proposes that development is not dictated by a hard-wired genetic program; instead, the organism responds to the surrounding environment and the risk of many diseases is set during this time. It has become increasingly evident that there is an important role for environmental factors in the onset of complex conditions such as allergic diseases and that the role of fixed
generic variation is far less than previously believed. Therefore, new approaches toward disease prevention with an emphasis on early interventions, that is, preconception and/or during pregnancy, need to be widely investigated. Current evidence suggests that the role of maternal diet during pregnancy in subsequent disease development is a priority area for future studies, because many of the immune modulatory processes may start in utero.

The role of environmental and lifestyle factors in developing allergies has been examined in a number of epidemiological studies. A systematic review has investigated the association of nutrient deficiencies with the risk of development of asthma and allergic diseases in children. This review included 62 observational studies and indicated that vitamins A, D, and E; zinc; fruits and vegetables; and a Mediterranean diet during pregnancy may prevent asthma and wheezing. However, this review was based on observational studies, which carry a high risk of bias, and there is a need for secondary research based on summary of more robust intervention studies.

The purpose of this systematic review was to summarize the existing evidence from randomized controlled trials (RCTs) for the association between intake of vitamin supplements during pregnancy and the risk of developing allergic disorders in the offspring.

METHODS
Criteria for considering studies for this review
Types of studies. Only RCTs (including cluster RCTs and quasi-RCTs) with a minimum follow-up of 1 month postnatally were included. The review considered studies that documented clinical outcome data and used any type of vitamins. No language restriction was applied.

Types of participants. Pregnant women and their offspring, regardless of their location, were considered as the target group for this systematic review. High-risk populations were not excluded.

Types of interventions. Studies that used any vitamin supplementation during pregnancy, irrespective of dose, formulation or mode of delivery, and composition, for example, oil and tablet, were included.

Trials were also included if the intervention(s) had been extended after pregnancy either during breast-feeding or with the infants or both.

Outcomes of interest. Trials were included if they had reported clinical outcomes of allergy in the offspring, either as a primary end point or as a secondary end point. Allergic outcomes were defined as asthma, wheeze, rhinitis, eczema, food allergy, and positive skin prick test result (to any allergen) and elevated specific IgE level. Outcomes included were those that used a validated method as opposed to parental reports.

Search strategy for identification of studies. A comprehensive search strategy, including all the relevant synonyms for the main concepts, was developed covering the main bibliographic databases (see this article’s Online Repository at www.jaci-inpractice.org). Trials were identified through systematic searches within 3 main electronic databases, as advised by the Cochrane collaboration:

a. Cochrane Library (current issue) including the following:
   • Cochrane Database of Systematic Reviews (CDSR)
   • CENTRAL (trials)
   • Database of Reviews of Effectiveness
b. MEDLINE (EBSCOhost)
c. SCOPUS

When searching MEDLINE, the subject-specific terms were combined with the Cochrane Highly Sensitive Search Strategy for identifying randomized trials in MEDLINE: sensitivity-maximizing version. We adapted the primary search strategy for MEDLINE (EBSCOhost) for use in the other databases when relevant. The last search for literature was conducted in January 2016.

The clinical trials registry and World Health Organization platforms were searched for ongoing and recently completed trials. Conference proceedings were identified through the Institute for Scientific Information Web of Science, and the British Library E-Theses Online Service was searched for retrieving theses. No language or publication status restrictions were imposed. References of included studies were crosschecked for additional studies.

Data collection and analysis
Selection of studies. The main reviewer (M.V.) screened all the search results against the eligibility criteria and all those that were clearly irrelevant were excluded from further consideration. Thereafter, a tailored eligibility form was used by M.V. to appraise the retrieved studies, abstract, and full text for relevance against the full inclusion criteria. Where there was uncertainty about the inclusion of a particular study, other members of the review team (H.M. and T.D.) were consulted and a consensus was reached about the study eligibility. All the included studies were discussed and approved by the review team.

Data extraction. M.V. extracted the data using a tailored data extraction form (Table E1, available in this article’s Online Repository at www.jaci-inpractice.org). Detailed information on study characteristics was recorded. Throughout the data extraction process, any disagreements about the interventions and outcomes were discussed and resolved within the review team. There was no blinding to the name of authors, institutions, journals, or the outcomes of the trials during the process. Ten percent of all the extracted data was randomly selected and double checked by a second reviewer (H.M.) for accuracy against the trial reports.

Assessment of risk of bias in included studies. The risk of bias tool described in the Cochrane Handbook for Systematic Reviews for Interventions was used to appraise the studies. The tool includes 7 domains: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessments, incomplete outcome data, selective outcome reporting, and other bias.

Measurement of treatment effect
Dichotomous data were analyzed as relative risk (RR) with 95% CI and continuous data as mean difference or standardized mean difference, with 95% CI.
Unit of analysis issues. In trials with more than 1 intervention arm, multiple pairwise comparisons of intervention groups versus comparator were avoided. Therefore, data from different intervention arms were pooled for an overall comparison with the control group or the placebo arm. The weight assigned to the control group was considered as the total number of participants in the comparator group versus the total number of participants in the combined intervention arms.6

Handling missing data. All the relevant reported information for the number of missing participants was extracted and if undocumented, this was incorporated into the assessment of risk of bias. No imputed techniques were used for retrieving missing data.

Assessment of heterogeneity. We used visual inspection of Forest plots and also the chi-square test to measure statistical heterogeneity between effects sizes of included studies ($I^2 < .05$).19 $I^2$ statistics were used to quantify the amount of possible variability in effect estimates that is due to heterogeneity rather than chance ($I^2 > 30\%$, moderate heterogeneity; $I^2 \geq 75\%$, considerable heterogeneity).

Assessment of reporting biases. Every effort was made to identify unpublished studies through searching abstracts and ongoing trials databases. Publication bias was assessed using funnel plots.15 The asymmetry was assessed visually in the plots and no formal statistical tests were conducted. The funnel plot was helpful to explore possible small study biases for some of the primary outcomes (see this article’s Online Repository at www.jaci-inpractice.org).

Data synthesis. We used Epi Reviewer version 4.4.3.0. for conducting meta-analyses using random-effects model. Dichotomous data were entered as events and the number of participants. Data were pooled using the random-effects model where heterogeneity was reported as 75% or less.3 We also reported RR as a statistical choice in conducting the meta-analyses because it is easy to interpret.12

Subgroup analysis and investigation of heterogeneity. We performed subgroup analyses on the basis of type of vitamin and type of control group (i.e., placebo vs no treatment).

Sensitivity analysis. We did not conduct any sensitivity analysis because of the small number of studies that contributed to meta-analyses.

RESULTS
The results of the search strategy yielded 341 studies, of which 26 were selected for full-text assessment (Figure 1). We included
<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th>Age at Start of Treatment</th>
<th>Gender</th>
<th>No. of Participants</th>
<th>Method</th>
<th>Intervention</th>
<th>Duration</th>
<th>Main Findings</th>
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<tr>
<td>A</td>
<td>USA</td>
<td>3-7 yrs</td>
<td>Boys</td>
<td>249</td>
<td>RCT</td>
<td>HEA</td>
<td>1 year</td>
<td>Lower incidence of asthma symptoms</td>
</tr>
<tr>
<td>B</td>
<td>UK</td>
<td>5-11 yrs</td>
<td>Girls</td>
<td>120</td>
<td>RCT</td>
<td>HEA, BM</td>
<td>1 year</td>
<td>Decreased bronchodilator use</td>
</tr>
<tr>
<td>C</td>
<td>Japan</td>
<td>2-7 yrs</td>
<td>Boys</td>
<td>180</td>
<td>RCT</td>
<td>HEA, BM</td>
<td>1 year</td>
<td>Improved pulmonary function</td>
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<tr>
<td>D</td>
<td>Canada</td>
<td>4-12 yrs</td>
<td>Girls</td>
<td>300</td>
<td>RCT</td>
<td>HEA</td>
<td>1 year</td>
<td>No significant difference in outcomes</td>
</tr>
</tbody>
</table>

HEA: High-dose Elemental Asthma
BM: Baseline Management

Note: This table is a summary of findings from various studies investigating the effectiveness of high-dose elemental asthma treatment compared to baseline management. The table includes information on study design, population demographics, intervention methods, and main findings.
5 RCTs comparing at least 1 vitamin with a control that met the inclusion criteria for this systematic review.

These included trials (including a total of 2456 children) were represented by 5 original articles1,15 and 6 grouped as their companion articles.16,9,25 Table I presents the characteristics of the included trials, their companion articles, and the study population. The trials were conducted in the United Kingdom, Denmark, and the United States. The types of vitamin supplements included were vitamins C + E, vitamin D,16,9,25 and crushed vitamin C.16,9 The duration of intervention and follow-up in the included studies varied from 3.5-4 to 7.5 months and 12 to 36 months, respectively. In trials that used vitamin C and vitamins C + E, a higher blood concentration of vitamins was observed in those assigned antioxidants.16 In trials that used vitamin D, level of maternal 25-hydroxyvitamin D measured either at third trimester or after delivery was significantly higher in the treatment group versus the comparison group.16,9,25

The most frequently reported outcomes were wheeze and eczema. As expected with systematic reviews, there were differences between trials in terms of the type of the population, the supplementation used, and the comparators. We have therefore described the results of individual studies narratively and conducted meta-analysis only when there was no evidence of statistical heterogeneity. The definition and diagnosis method of the outcomes in each study are presented in Table E1, available in this article's Online Repository at www.jaci-inpractice.org.

Vitamin C studies

Greenough et al’s (2010) study. The study was conducted in the United Kingdom between August 2003 and June 2007.15 The studied sample was pregnant women at risk of developing preclampsia. Women were supplemented with daily vitamin C (1000 mg) tablets and vitamin E (400 IU) gelatin capsules, from 16 to 22 gestation weeks until delivery. Women in the control group received identical tablets of microcrystalline cellulose with addition of tartaric acid and citric acid along with gelatin capsules of sunflower seed oil. Compliance with the intervention was measured by counts of returned pills. Primarily, this study was designed to prevent the risk of fetal growth restriction and premature delivery in the women15 and the extended follow-up at 2 years has assessed the effect of the vitamin intervention on respiratory outcomes in children.

The list of the reported outcomes in the study is presented in Table I. The outcomes of “asthma” and “eczema” are reported at age 1 year and “recurrent wheeze” at age 2 years. No statistically significant association was observed between the intervention and control groups for prevention of recurrent wheeze (10 of 386 vs 11 of 366; odds ratio [OR], 0.83; 95% CI, 0.26-2.59; P = .66) and asthma (23 of 386 vs 23 of 366; OR, 0.94; 95% CI, 0.42-2.11; P = .85). In addition, the results did not show a significant association between prenatal intake of vitamins C + E and prevention of eczema (98 of 386 vs 86 of 366; OR, 1.10; 95% CI, 0.70-1.74; P = .58).

McEvoy et al’s (2014) study. The study was conducted in the United States between March 2007 and January 2011.11 The studied sample was smoking pregnant women. Women were supplemented with daily crushed vitamin C (500 mg) gel capsules, from 22nd gestation week until delivery. Women in the control group received ground cornstarch in gel capsules. Adherence was measured by dividing the number of capsules taken by the total number prescribed in a given period.

The study reported the efficiency of consumption of vitamin C during pregnancy on pulmonary function tests and wheezing in children at age 1 year. The list of the reported outcomes in the study is presented in Table I. The results of the unadjusted analysis showed no significant statistical association between the intervention and control groups for outcome measure defined as “recurrent wheeze” (9 of 76 vs 17 of 86; OR, 0.56; 95% CI, 0.27-1.18; P = .13). A significant difference was observed for the outcome of “at least 1 episode of wheezing” between the intervention and control groups (15 of 76 vs 31 of 83; OR, 0.56; 95% CI, 0.33-0.95; P = .03).

Given the fact that there is high heterogeneity between the studies that supplemented pregnant women prenatally with vitamin C, we did not perform meta-analysis for these trials.

Vitamin D studies

Goldring et al’s (2013) study. The study was conducted in the United Kingdom between April and November 2007.18 This study recruited pregnant women with multiple ethnicities. The study introduced 2 intervention arms, as women were randomized either to receive a daily dose of ergocalciferol (800 IU) or a single oral dose of cholecalciferol (200,000 IU, bolus), from 27 gestation weeks until delivery. The comparator in this study was defined as “no treatment.” Adherence was measured by telephone calls during pregnancy.

This study followed-up children to age 5 years and this systematic review only reports the results for the intervention arm of daily vitamin D. The results of unadjusted analysis for “recurrent wheezing” showed no statistically significant association between prenatal intake of daily vitamin D and the control group (8 of 56 vs 7 of 50; OR, 1.02; 95% CI, 0.40-2.61; P = .97). Furthermore, no significant association was observed for the outcome measure of “wheeze with positive asthma predictive index” (6 of 56 vs 7 of 50; OR, 0.77; 95% CI, 0.28-2.13; P = .61) between the study arms. The outcomes of “eczema in the last year” (11 of 55 vs 7 of 49; OR, 1.40; 95% CI, 0.59-3.33; P = .44) and “food allergy diagnosis” (8 of 55 vs 3 of 49; OR, 2.38; 95% CI, 0.67-8.46; P = .16) did not show a significant statistical association for the prenatal consumption of daily vitamin D in comparison to control.

Chawes et al’s (2016) study. The study was conducted in Denmark between 2008 and 2010.19 The studied sample was unselected pregnant women. Women were supplemented with daily vitamin D3 (2400 IU) tablets, from 24 gestation weeks to 1 week after delivery. Women in the control arm received tablets containing no active substance. In addition, women assigned to both intervention and control arms received an extra 400-IU dose of vitamin D3 as part of their routine care. Compliance to the intervention was measured by counts of returned pills.

The study reported the cumulative incidence of allergic outcomes by age 3 years. The results of unadjusted analysis indicated that the risk of developing recurrent wheeze did not show a significant difference between the intervention and control groups (47 of 295 vs 57 of 286; hazard ratio [HR], 0.76; 95% CI, 0.52-1.12; P = .16). Asthma was reported at age 3 years only and no significant difference was observed between the intervention and control groups (32 of 278 vs 47 of 271; OR, 0.82; 95% CI, 0.50-1.36; P = .45). Furthermore, there was no
significant statistical difference between the study arms for eczema as an outcome (68 of 295 vs 72 of 286; HR, 0.90; 95% CI, 0.65-1.26; P = .55). Children in the intervention arm reported statistically significant “lower episodes of troublesome lung symptoms” compared with the control group (5.9 vs 7.2; incidence rate ratio (IRR), 0.83; 95% CI, 0.71-0.97; P = .02).

The cumulative results for skin prick test and specific IgE outcomes were not statistically different between the intervention and control groups (24 of 294 vs 19 of 283; OR, 1.24; 95% CI, 0.66-2.31; P = .51 and 34 of 289 vs 22 of 278; OR, 1.53; 95% CI, 0.89-2.63; P = .13, respectively).

Litonja et al’s (2016) study. The study was conducted in the United States between 2009 and 2011.17 The study sample was women with a history of atopy. Women were supplemented with daily vitamin D₃ (4000 IU) tablets, between 10 and 18 gestation weeks until delivery. The nature of the placebo capsules was not reported. Women in both study arms also received a multivitamin with 400 IU of vitamin D. Adherence to the intervention was measured by electronic medication container caps.

The study reported the cumulative incidence of allergic outcomes by age 3 years. The outcomes of “asthma or recurrent wheeze” were reported together and the results showed no significant statistical difference between the intervention and control groups (58 of 405 vs 120 of 401; HR, 0.8; 95% CI, 0.6-1.0; P = .051). There was also no significant statistical difference in the risk of developing “eczema with rash” in the study arms (83 of 405 vs 89 of 401; HR, 0.9; 95% CI, 0.9-1.3; P = .36). The results for positive specific IgE test results at 3 years showed a significant statistical difference between the intervention and control groups (43 of 405 vs 50 of 401; mean difference (MD), −1.7; 95% CI, −3.4 to 0.0; P = .02).

Meta-analyses of vitamin D studies
We conducted a meta-analysis for the outcome measure of “recurrent wheeze” for trials that used vitamin D prenatally in pregnant women. Figure 2 shows the forest plot for this outcome. Three trials contributed to the meta-analysis including a total of 1493 children. No statistically heterogeneity was observed between the included trials ($I^2 = 0.16; P = .92; P = 0.00$) (Figure 3). The results of the present meta-analysis showed an association between the maternal intake of daily vitamin D during pregnancy and a lower risk of developing recurrent wheeze in offspring (RR, 0.812; 95% CI, 0.673-0.98). We also conducted the meta-analysis including only the 2 recent vitamin D trials15,17 and it yielded similar results (Forest plot not shown).

Risk of bias in included trials
The risk of bias figures and authors’ judgments are presented in Table E5, available in this article’s Online Repository at www.jaci-inpractice.org. Only 1 trial was deemed to have a low risk of bias across all domains.15 Of the 5 trials, most had adequate random sequence generation (n = 3), allocation concealment (n = 3), and performance bias (n = 3). All trials were rated as having a low risk of bias for blinding of outcome assessment and selective outcome reporting. Completeness of outcome data was rated as having high risk of bias for 1 trial15 because the study had a high loss to follow-up and the authors acknowledged the fact that the study was an unplanned extended follow-up of the original trial for measuring allergic outcomes in children. The original trial was primarily designed to assess the effect of
vitamins C and E supplementation on developing preeclampsia in women at increased risk.

DISCUSSION

This is the first systematic review of RCTs that investigated the association of prenatal intake of vitamins with the risk of developing allergic/respiratory diseases in the offspring. We identified 5 RCTs with a total of 2456 children. The studies were of unselected pregnant women, women with a history of atopy, pregnant women at risk of developing preeclampsia, different ethnic/race groups, and smoking pregnant women. Two studies were judged to have a high risk of bias due to their performance bias or a high rate of loss to follow-up. All trials were rated as having a low risk of bias for blinding of outcome assessment. It was not possible to conduct meta-analyses for vitamin C studies due to observed differences between the included trials. Maternal vitamin D consumption during pregnancy was associated with a lower risk of developing recurrent wheeze in offspring, when compared with placebo/control. However, we were not able to investigate the effect of vitamin D on other allergic outcomes because outcomes were reported differently in the included trials. In all trials, supplementation with vitamins significantly increased the concentration of vitamins in the intervention group compared with the control group by the end of the intervention.

Observational studies typically report a beneficial effect of higher intake of vitamin D as well as antioxidants during pregnancy on allergic outcomes. The results from this systematic review proposed a protective effect of prenatal intake of vitamin D during pregnancy for prevention of recurrent wheeze in offspring. However, we could not address the effect of prenatal intake of vitamin C or D on other allergic outcomes owing to the observed heterogeneity between the trials.

It is possible that the follow-up periods of the studies for this review have been too short to detect other allergic outcomes, that is, asthma. For example, wheezing is known as a primary symptom of asthma in early childhood and about 40% of childhood wheeze will persist later in life and will eventually develop into asthma by age 6 years, indicating that majority of cases of wheeze during infancy are in fact acute respiratory infection. Therefore, extended follow-up of these trials could help to provide a clearer answer as to whether the vitamin D intervention is beneficial for asthma prevention.

There were also some limitations in the studies’ design. For example, the trials were statistically underpowered to detect an effect for their primary and/or secondary outcome measures. Significant differences were observed for only some of the secondary outcomes as ‘at least 1 episode of wheezing,’ episodes of troublesome lung symptoms, and ‘positive specific IgE’ and trials failed to show a beneficial effect for primary allergic outcomes such as wheeze and asthma in children. Also, the trials used different doses of vitamins during pregnancy. The dose of vitamin D varied between 800 and 4000 IU and doses of vitamin C and/or E varied between 500 and 1000 mg. It is possible to hypothesize that lower doses of vitamins may have failed to reach the desirable level of 25-hydroxyvitamin D or antioxidants in pregnant women to have an influential effect on the fetal immune programming and lung function. However, this is refuted by studies that have reported similar effect size using higher doses of vitamin D. A previous RCT by addressing the safety and efficacy of vitamin D supplementation during pregnancy showed that a 4000-IU dose of vitamin D is a safe approach and was necessary to optimize the circulating concentration of 25-hydroxyvitamin D levels to 80 nmol/L, or more. There is limited evidence on the safety of intake of vitamins C and E at any stage of pregnancy; however, the Institute of Medicine’s Food and Nutrition Board has set an upper limit of 2000 mg and 1000 mg per day for the ingestion of vitamins C and E, respectively, during pregnancy in the United States. Furthermore, in all trials, the intervention was started in the second trimester in pregnancy. However, the development of the lungs begins in the first trimester in pregnancy and vitamin D plays an immunomodulatory role in the development of lung and immune system. Therefore, the interventions might have commenced too late in pregnancy or some used too low doses of vitamin D to have a beneficial impact on lung development. Finally, the studies recruited different types of population, which limits the generalizability of the studies. Baseline levels of vitamin D vary in different geographical areas and this issue has not been addressed in the conducted trials. Well-designed trials are necessary to address all these possible confounders among different populations. Further larger scale research should administer vitamin D earlier in pregnancy or propregnancy and use appropriate doses of vitamin D to achieve a desirable level of vitamin D in maternal and fetal blood. Furthermore, studies assessing the efficiency of nutrients are required to consider the defined guidelines in their clinical design, enabling to test the associated hypothesis in a valid manner.

To date, no other systematic review has evaluated the effect of prenatal vitamins on the prevention of allergic and/or respiratory outcomes in children. The result from the current evidence is promising that prenatal intake of vitamin D could protect childhood wheeze. The role of maternal consumption of vitamins during pregnancy in the risk of developing other allergic outcomes and sensitization needs to be investigated in larger well-designed trials. Furthermore, it will be important for future research to examine the impact of the timing of the intervention and the optimum dose of vitamins. We were unable to perform any meta-analyses on the timing or dose of intervention and study populations due to the small number of trials that could contribute to meta-analyses.

The current evidence suggests that prenatal intake of daily vitamin D might protect against recurrent childhood wheeze; however, there is currently lack of evidence that prenatal intake of vitamins can prevent any other allergic/respiratory outcomes.

REFERENCES

RESEARCH QUESTION USING PATIENT INTERVENTION COMPARISON OUTCOME STUDY STRUCTURE AND SEARCH STRATEGY

The following 4 important concepts were identified on the basis of the research question:

- Population: Pregnancy
- Intervention: Vitamins and/or dietary supplements
- Outcome(s): Allergic outcomes
- Study: Randomized controlled trial

Various keywords and synonyms were identified using the Patient Intervention Comparison Outcome Study approach. A full list of the used keywords for each concept is presented below.

Within groups of terms, the terms were combined using OR and the groups of terms themselves were then combined in the following manner: #1 AND #2 AND #3 AND #4.

Search strategy

1. pregnan* OR ((antenatal or ante-natal or ante natal)) OR ((prenatal or pre-natal or pre nat al)) OR ((mother or maternal))
2. (MH "Pregnancy+-")
3. vitamin* OR (supplement* or dietary supplement*) OR nutrient* OR mineral* OR micronutrient*
4. vitamins (MeSH terms)
5. (allerg* or atopy or dermatitis or eczema) OR (asthma or asthma prevention) OR (wheez*) OR (food hypersensitivity or food allergy or food intolerance) OR (non-IgE or non-IgE mediated allerg*) OR (hypersensitivity or sensitisation) OR rhinitis
6. (MH "Hypersensitivity+-")
7. (randomised controlled trial or randomised control trial or random controlled study or randomised clinical trial or controlled clinical trial or controlled trial or random allocation) OR (single blind or single blind method or single blind trial or double blind method OR double-blind method) OR (clinical trial* or quasi-experimental study or placebo-controlled trial or placebo control or intervention study or follow-up study*)
8. (MH "Clinical Trials as Topic+-") OR (MH "Controlled Clinical Trials as Topic+-")
9. (#1 OR #2) AND (#3 OR #4) AND (#5 OR #6) AND (#7 OR #8)

FIGURE E1. Funnel plot for vitamin D versus placebo or no treatment for prevention of recurrent wheeze in offspring.
<table>
<thead>
<tr>
<th>Short Title</th>
<th>Random Sequence Generation</th>
<th>Allocation Concealment</th>
<th>Double Blinding</th>
<th>Blinding of Outcome Assessment</th>
<th>Incomplete Outcome Data</th>
<th>Selective Outcome Reporting</th>
<th>Other Sources of Bias</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grimshaw (2003)</td>
<td>?</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
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<td>Gelling (2013)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>McEvoy (2014)</td>
<td>?</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Oconnor (2016)</td>
<td>+</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>?</td>
</tr>
<tr>
<td>Litinaria (2016)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Random Sequence Generation: 60% [Green] - 40% [Red]
Allocation Concealment: 60% [Green] - 40% [Red]
Double Blinding: 60% [Green] - 40% [Red]
Blinding of Outcome Assessment: 100% [Green] - 0% [Red]
Incomplete Outcome Data: 100% [Green] - 0% [Red]
Selective Outcome Reporting: 100% [Green] - 0% [Red]
Other Sources of Bias: 60% [Green] - 40% [Red]

Low risk of bias: [Green]  Unclear risk of bias: [Yellow]  High risk of bias: [Red]

**FIGURE E2.** Summary of risk of bias assessment.
TABLE E1. Data extraction tool

<table>
<thead>
<tr>
<th>1. Exclude nonrandomized</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. Study protocol</td>
</tr>
<tr>
<td>3. Linked record-Do not obtain data</td>
</tr>
<tr>
<td>4. Study details</td>
</tr>
<tr>
<td>Country</td>
</tr>
<tr>
<td>Recruitment period</td>
</tr>
<tr>
<td>Setting</td>
</tr>
<tr>
<td>Informed consent</td>
</tr>
<tr>
<td>Ethical approval</td>
</tr>
<tr>
<td>Source of funding</td>
</tr>
<tr>
<td>5. Trial type</td>
</tr>
<tr>
<td>PC-RCT</td>
</tr>
<tr>
<td>Randomized controlled trial-placebo controlled</td>
</tr>
<tr>
<td>RCT</td>
</tr>
<tr>
<td>Randomized controlled trial</td>
</tr>
<tr>
<td>Quasi experimental</td>
</tr>
<tr>
<td>No. &amp; name of study groups/arms</td>
</tr>
<tr>
<td>ITT</td>
</tr>
<tr>
<td>Intention-to-treat analysis</td>
</tr>
<tr>
<td>6. Study sample</td>
</tr>
<tr>
<td>Matched baseline characteristics</td>
</tr>
<tr>
<td>Women's age</td>
</tr>
<tr>
<td>Exclusion criteria</td>
</tr>
<tr>
<td>FH of atopy</td>
</tr>
<tr>
<td>Unselected sample</td>
</tr>
<tr>
<td>Subjects are taken from an unselected population</td>
</tr>
<tr>
<td>No. of participants at randomization</td>
</tr>
<tr>
<td>No. at F-U</td>
</tr>
<tr>
<td>Missing participants</td>
</tr>
<tr>
<td>Reasons missing</td>
</tr>
<tr>
<td>Time points measured</td>
</tr>
<tr>
<td>Earlier F-U(s) reported time points</td>
</tr>
<tr>
<td>Infant’s age at last F-U</td>
</tr>
</tbody>
</table>

7. Intervention
- Vitamin supplement type
  - Vitamin C
  - Vitamins C & E
  - Vitamin D (cholecalciferol)
  - Vitamin D (ergocalciferol)
  - Combined vitamin D
- Daily dosage
- Comparisons
  - Placebo
  - No treatment
- When Int. has been applied?
  - Pregnancy alone
  - Pregnancy & after delivery
- Timing in pregnancy
- Duration of INT. in pregnancy
- Total duration of INT.
- Side effects
- Compliance

8. Outcomes
- The defined and measured end point(s) in the study
- Allergic disease(s)
- Wheeze

(continued)

TABLE E1. (Continued)

<table>
<thead>
<tr>
<th>9. Diagnosis method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Please tick all that apply</td>
</tr>
<tr>
<td>F-U questionnaires, eg. ISAAC</td>
</tr>
<tr>
<td>Clinician diagnosed</td>
</tr>
<tr>
<td>SPT</td>
</tr>
<tr>
<td>If the test is conducted vs any specific allergen, eg. egg-peanut-milk, please record the details and mean diameter of the wheal, eg. 2.1 mm</td>
</tr>
<tr>
<td>Self report</td>
</tr>
<tr>
<td>Physical examination</td>
</tr>
<tr>
<td>Specific IgE</td>
</tr>
</tbody>
</table>

F-U: Follow-up; Int.: intervention; ISAAC: International Study of Asthma and Allergies; SPT, skin prick test.
### TABLE E2. Definition of outcomes and diagnostic criteria in individual studies

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Greenough et al.'s study (2010)</td>
<td>Asking women whether or not their infant had coughed and/or wheezed and the frequency of the cough and wheeze</td>
</tr>
<tr>
<td>Asthma: chest symptoms in first 12 mo</td>
<td>Non-defined</td>
</tr>
<tr>
<td>Eczema, in first 12 mo</td>
<td>Non-defined</td>
</tr>
<tr>
<td>McEvoy et al.'s study (2014)</td>
<td>By respiratory questionnaire (pediatric version) to the infant's primary caretaker asking medication for wheezing</td>
</tr>
<tr>
<td>Medication for wheezing, through age 1 y</td>
<td>By respiratory questionnaire (pediatric version) to the infant's primary caretaker asking for presence or absence of wheezing, maternal smoking, and exposure to second-hand smoke</td>
</tr>
<tr>
<td>At least 1 episode of wheezing, through age 1 y</td>
<td></td>
</tr>
<tr>
<td>Goldring et al.'s study (2013)</td>
<td>Recurrent wheezing (≥2 episodes of reported wheezing since birth), defined by ISAAC criteria</td>
</tr>
<tr>
<td>Wheeze with positive asthma predictive index</td>
<td>Using loose criteria</td>
</tr>
<tr>
<td>Chaves et al.'s study (2016)</td>
<td>Persistent wheeze (0-3 y)</td>
</tr>
<tr>
<td>Persistent wheeze, as primary end point, was diagnosed according to a previously validated quantitative algorithm, requiring all of the following: (1) recurrent wheeze (verified daily recordings of ≥5 episodes of troublesome lung symptoms [cough, wheeze, and/or dyspnea] lasting ≥3 d within 6 mo), (2) typical symptoms of asthma (e.g., exercise-induced symptoms, prolonged nocturnal cough, or persistent cough outside common cold), (3) need for intermittent bronchodilator, and (4) response to a 3-mo trial of inhaled corticosteroids and relapse upon cessation</td>
<td></td>
</tr>
<tr>
<td>Asthma, 3 y</td>
<td>Asthma, as secondary end point, was diagnosed in children fulfilling the persistent wheeze criteria at age 3 y</td>
</tr>
<tr>
<td>Eczema, 0-3 y</td>
<td>Eczema was diagnosed according to the criteria of Hanifin and Rajka including typical morphology and localization of skin lesions</td>
</tr>
<tr>
<td>Specific IgE, 0-3 y</td>
<td>Allergic sensitization was diagnosed at 6 and 18 mo by specific IgE level of 0.35 kU/L or higher against raw milk, pasteurized eggs, dogs, or cats</td>
</tr>
<tr>
<td>Litonjua et al.'s study (2016)</td>
<td>Parental report of physician’s diagnosis of asthma was taken directly from the offspring questionnaire administered every 3 mo. Recurrent wheeze was defined by the occurrence of at least 1 of the following 5 conditions: (1) parental report of wheeze after child’s second birthday preceded by at least 1 report of wheeze before second birthday; (2) report of child’s use of asthma controller medication (defined as report of use of inhaled steroids or nebulizers, leukotriene modifiers, or steroid pills or liquids) after the second birthday, preceded by a report of wheeze before the second birthday; (3) 2 or more distinct parental reports of wheeze after the second birthday; (4) at least 1 parental report of wheeze and use of asthma controller medications at distinct visits, both subsequent to the second birthday; or (5) 2 distinct reports of use of asthma controller medications after the second birthday.</td>
</tr>
<tr>
<td>Asthma or recurrent wheeze in the first 3 y of life</td>
<td>Parental report of physician’s diagnosis of asthma or wheeze in typical distribution</td>
</tr>
<tr>
<td>Eczema with rash, 0-3 y</td>
<td>Parental report of physician’s diagnosis of eczema with rash in typical distribution</td>
</tr>
<tr>
<td>Positive specific IgE test results</td>
<td>Allergen sensitization (specific IgE to a panel of allergens)</td>
</tr>
<tr>
<td>Study author/ year</td>
<td>Random sequence generation</td>
</tr>
<tr>
<td>-------------------</td>
<td>----------------------------</td>
</tr>
<tr>
<td>Grennough et al. (2008)</td>
<td>Unclear</td>
</tr>
<tr>
<td>Ghring et al. (2013)</td>
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<tr>
<td>McEvoy et al. (2014)</td>
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</tr>
<tr>
<td>Chaves et al. (2016)</td>
<td>Low</td>
</tr>
<tr>
<td>Livi et al. (2016)</td>
<td>Low</td>
</tr>
</tbody>
</table>

F-U: Follow-up; LC-PUFA: long-chain polyunsaturated fatty acid.
Appendix 3.1: The protocol for systematic reviews

Systematic review of maternal dietary interventions during pregnancy and the risk of developing allergic disorders and obesity in offspring from birth to 18 years of age

Mariam Vahdaninia, Tara Dean, Heather Mackenzie

Citation
Mariam Vahdaninia, Tara Dean, Heather Mackenzie. Systematic review of maternal dietary interventions during pregnancy and the risk of developing allergic disorders and obesity in offspring from birth to 18 years of age. PROSPERO 2015:CRD42015024397 Available from: http://www.crd.york.ac.uk/PROSPERO/?view=record&ID=CRD42015024397

Review question(s)
To assess the effectiveness of dietary interventions during pregnancy on the prevention of allergic disorders in offspring.

To assess the effectiveness of dietary interventions during pregnancy on the prevention of obesity in offspring.

Searches
The following databases will be searched:

a. Cochrane Library

b. MEDLINE (EBSCOHOST)

c. SCOPUS

d. ISI Web of Science (Thomson Web of Knowledge) (conference proceeding)

b. Clinicaltrials.gov

c. WHO International Clinical Trials Registry Platform (ICTRP)

d. EThoS

Studies will be included from across the world with no limit to language or publication year.

Additional details of the search strategy can be found in the attached PDF document.

Types of study to be included
Only randomised controlled trials (including cluster randomised controlled trials) and quasi-randomised controlled trials will be eligible for inclusion.

The review will look for published reports of studies, which documented clinical outcome data and have a follow-up (F-U) period of at least one month. We will also look for unpublished data, where appropriate.

Condition or domain being studied
1. Clinical outcomes of allergy, either as primary or secondary endpoint, in the offspring from infancy period to adulthood. The primary outcome measure is defined as allergic diseases including infantile wheeze and only validated assessment of outcomes will be considered.

2. Obesity-related outcomes e.g. adiposity ratio, BMI. Only validated assessment of outcomes will be considered.
Participants/population
Pregnant women, across the world from general population, and their offspring.

Intervention(s), exposure(s)
We will include studies reporting one or more of the following interventions during pregnancy:

a. Food-based dietary advice (promoting a healthy diet) or food-based nutrient interventions
b. Multivitamins, supplementation and minerals
c. Fatty acids supplementation
d. Pre/Probiotics supplementation

Trials will also be included if the intervention(s) have been extended after pregnancy either in breast-feeding mothers, the infants or both.

The interventions may have also been applied in combination with an exercise/physical activity approach. This will include supervised exercises with a professional, a trained non-professional or volunteer and the provision of recommended exercises without supervision. However, exercise/physical activities as an intervention, without an accompanying dietary intervention, will not be the focus of this review. Studies focusing solely on exercise/physical activity interventions will not be included.

Comparator(s)/control
The comparators of interest will include routine usual diet, placebo or sham interventions and physical activity alone during pregnancy.

Outcome(s)
Primary outcomes
Allergic disorders: asthma, wheeze, eczema, positive skin prick test, positive specific IgE

Obesity: body mass index, adipose ratio, body composition.

We will scan the studies that have reported the outcome in children under the umbrella of a general term i.e. infant’s morbidity and immune function, epigenetic states/programming in the infant immune system, measurement of immune factors in cord blood, offspring disease risk in adulthood. These studies will be considered if there is identifiable data for the clinical outcome of interest (allergy and obesity) for this systematic review. We will also search these studies for any further published work such as an updated report for the outcomes of interest for this systematic review. Trials that report only birth outcomes i.e. birth size, preterm birth, infant’s body composition at birth, fetal growth will be excluded. However, these studies will also be searched for any updated report on the outcomes of interest in this systematic review.

Dichotomous data will be analysed as risk ratios or relative risk (RR) with 95% confidence intervals and continuous data as mean difference or standardised mean difference, with 95% confidence intervals (CI).

Secondary outcomes
Allergic disorders: rhinitis, food allergy, anaphylaxis, angioedema.

Obesity: obesity in offspring, offspring growth.

Data extraction, (selection and coding)
A tailored form will be used to extract relevant data from the included studies. The main reviewer (MV) will extract the following baseline study characteristics from the included studies:

a. Study details: country, recruitment period, setting, ethics, informed consent and funding body
b. Trial type: details of trial design, No. & name of study groups/arms

c. Study sample: comparability of groups, women's age, risk of atopy in the studied sample, No. at randomisation, No. at F-U, No. of missing participants, reasons for missing, time points measured, length of F-U

d. Intervention/comparison: detailed information about type of probiotics/fatty acids/vitamins/food avoidance, timing in pregnancy, mode of intervention delivery during pregnancy and/or infancy, total duration of intervention, side effects AND detailed information about comparison used e.g. type, mode of delivery

e. Reported outcomes: all the reported clinical allergic end points, either as primary or secondary outcome, with the relevant definition e.g. point prevalence, cumulative, crude, adjusted, combination of some outcomes

f. Diagnosis method: all methods defined for measuring the reported outcomes, e.g. questionnaires, clinical and/or laboratory examination(s)

Throughout the data extraction process, any disagreements about the interventions and outcomes will be discussed and resolved within the review team. There will be no blinding to name of authors, institutions, journals or the outcomes of the trials during the process. Ten percent of all the extracted data will be double checked by a second reviewer (Heather Mackenzie; HM) for accuracy against the trial reports.

Risk of bias (quality) assessment

The quality of each included trial will be assessed by MV using the tool described in the Cochrane handbook for systematic reviews for intervention (Higgins et al., 2011). The design, conduct and analysis of the trial will be assessed using a three-point scale: low risk of bias, high risk of bias, unclear. Risk of bias will be addressed to the following domains:

a. Random sequence generation: was the allocation sequence adequately generated?

b. Allocation concealment: was allocation adequately concealed?

c. Blinding of participants and personnel: was knowledge of the allocated intervention adequately prevented throughout the study i.e. blinded?

d. Blinding of outcome assessment: was knowledge of the outcome assessment adequately prevented i.e. blinded?

e. Incomplete outcome data: were incomplete outcome data adequately addressed for every outcome?

f. Selective outcome reporting: were reports of the study free of selective outcome reporting?

g. Other bias: was the study free from any other problems that could put it at risk of bias e.g. comparability of control group at entry, industry funding?

Any disagreements will be discussed and resolved between two reviewers (MV & HM) and if required, by consensus with a third reviewer (Tara Dean, TD).

Strategy for data synthesis

Data will be described in tables and where possible (where there are sufficient numbers of studies utilising the same interventions and comparisons), data will be combined and meta-analyses be performed as described in the Cochrane Handbook of Systematic Reviews of Interventions.

Analysis of subgroups or subsets

No sub-group analyses are planned.

Dissemination plans

The results of the systematic review will be presented in relevant conferences and we will aim to publish a full paper for the systematic reviews, when time allows.
Contact details for further information
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School of Health Sciences and Social Work
University of Portsmouth
James Watson Building
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Portsmouth
PO1 2FR
mariam.vahdaninia@port.ac.uk

Organisational affiliation of the review
University of Portsmouth

http://www.port.ac.uk/school-of-health-sciences-and-social-work/

Review team
Ms Mariam Vahdaninia, PhD candidate, University of Portsmouth, SHSSW
Professor Tara Dean, Dean of the Science Faculty, University of Portsmouth
Dr Heather Mackenzie, Faculty of Science, SHSSW

Anticipated or actual start date
27 November 2014

Anticipated completion date
30 January 2016

Funding sources/sponsors
These reviews are part of my self-funded PhD study and I am based in School of Health Sciences and Social Work (SHSSW). I have been provided with some training opportunities offered and funded by the university.

Conflicts of interest
None known

Language
English

Country
England

Subject index terms status
Subject indexing assigned by CRD

Subject index terms
Diet; Female; Humans; Hypersensitivity; Obesity; Parturition; Pregnancy; Risk

Stage of review
Ongoing

Date of registration in PROSPERO

Page: 4 / 5
31 July 2015

Date of publication of this revision
31 July 2015

<table>
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<th>Completed</th>
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<tr>
<td>Piloting of the study selection process</td>
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<tr>
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<tr>
<td>Data analysis</td>
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</tr>
</tbody>
</table>

PROSPERO
International prospective register of systematic reviews
The information in this record has been provided by the named contact for this review. CRD has accepted this information in good faith and registered the review in PROSPERO. CRD bears no responsibility or liability for the content of this registration record, any associated files or external websites.
Appendix 3.2: Search strategy (allergic outcomes)

The following four important concepts were identified based on the research question:

- Pregnancy
- Diet
- Allergy (Non-communicable diseases)
- Randomised controlled trial

A variety of keywords and synonyms were identified using the Patient Intervention Comparison Outcome Study (PICOS) approach. A full list of the used keywords for each concept in the databases is presented in the following table.

Within groups of terms, the terms were combined using OR and the groups of terms themselves were then combined in the following manner: #1 AND #2 AND #3 AND #4.

Relevant citations and key authors were identified. To prevent bias, no restriction was placed on the year of publication or language.
### Search Log - MEDLINE (From inception-January 2015) (appendix 3.2)

<table>
<thead>
<tr>
<th>Search No.</th>
<th>Date of search</th>
<th>Concepts</th>
<th>Terms used</th>
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<th>Comments</th>
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<td>pregnan* OR ((antenatal or ante-natal or ante natal) ) OR ((prenatal or pre-natal or pre natal) ) OR ( (mother or maternal) )</td>
<td>942,914</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>9/12/2014</td>
<td></td>
<td>(MH &quot;Pregnancy&quot;)</td>
<td>714,997</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>9/12/2014</td>
<td></td>
<td>S1 OR S2 (#1 &amp; #2)</td>
<td>956,346</td>
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</tr>
<tr>
<td>4</td>
<td>9/12/2014</td>
<td>2nd filter</td>
<td>Diet* or (food or consumption or intake) OR (mineral or nutrition or nutrient) OR (milk or egg or peanut or fruit or vegetable) OR (vitamin* or fatty acid* or supplement*) OR (probiotic* or prebiotic*) OR folic acid</td>
<td>1,857,545</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>9/12/2014</td>
<td></td>
<td>(MH &quot;Nutritional Physiological Phenomena&quot;)</td>
<td>396,003</td>
<td></td>
</tr>
<tr>
<td>6</td>
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</tr>
<tr>
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<td>9/12/2014</td>
<td>3rd filter</td>
<td>non-communicable disease* OR noncommunicable disease* OR non communicable disease*</td>
<td>4,315</td>
<td>No MESH terms for non communicable diseases</td>
</tr>
<tr>
<td>8</td>
<td>9/12/2014</td>
<td></td>
<td>allerg* or atopy or dermatitis or eczema) OR (asthma or wheez*) OR (food hypersensitivity or food allergy or food intolerance) OR (non IgE or non-IgE or mediated allerg*) OR hypersensitivity OR rhinitis</td>
<td>433,069</td>
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</tr>
<tr>
<td>9</td>
<td>9/12/2014</td>
<td></td>
<td>(MH &quot;Hypersensitivity&quot;)</td>
<td>280,496</td>
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<tr>
<td>10</td>
<td>9/12/2014</td>
<td></td>
<td>S8 OR S9 (#8 or #9)</td>
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<tr>
<td>11</td>
<td>9/12/2014</td>
<td></td>
<td>S7 OR S10 (#7 or #10)</td>
<td>474,253</td>
<td></td>
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<tr>
<td>12</td>
<td>9/12/2014</td>
<td>4th filter</td>
<td>randomised controlled trial or randomised control trial or randomised controlled study or randomised clinical trial or controlled clinical trial or controlled trial or random allocation ) OR ( single blind or single blind method or single blind trial or double blind method OR double-blind method ) OR ( clinical trial* or quasi-experimental study or placebo-controlled trial or placebo control or intervention study or follow-up stud*</td>
<td>1,134,412</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>9/12/2014</td>
<td></td>
<td>(MH &quot;Clinical Trials as Topic&quot;) OR (MH &quot;Controlled Clinical Trials as Topic&quot;)</td>
<td>279,911</td>
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<tr>
<td>14</td>
<td>9/12/2014</td>
<td></td>
<td>S12 OR S13 (#12 or #13)</td>
<td>1,195,071</td>
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<tr>
<td>15</td>
<td>9/12/2014</td>
<td>combined</td>
<td>S3 AND S6 AND S11 AND S14</td>
<td>423</td>
<td>423</td>
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### Search Log - COCHRANE (From inception-January 2015) (appendix 3.2)

<table>
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<th>Date of search</th>
<th>Concepts</th>
<th>Terms used</th>
<th>Hits</th>
<th>Comments</th>
</tr>
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<tbody>
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<td>10/12/2014</td>
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<td>pregnan* or antenatal or ante-natal or ante natal or prenatal or pre-natal or pre natal or mother or maternal</td>
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<tr>
<td>2</td>
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<td>MeSH descriptor: [Pregnancy] explode all trees</td>
<td>#1 or #2</td>
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<td></td>
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<tr>
<td>3</td>
<td>10/12/2014</td>
<td>2nd filter</td>
<td>diet or food or consumption or intake or mineral or nutrition or nutrient or milk or egg or peanut or fruit or vegetable or vitamin* or fatty acid* or supplement* or probiotic* or prebiotic* or folic acid</td>
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<td>non-communicable disease* or non communicable disease* or noncommunicable disease*</td>
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<td></td>
</tr>
<tr>
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<td>MeSH descriptor: [Nutritional Physiological Phenomena] explode all trees</td>
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<td>6</td>
<td>10/12/2014</td>
<td>4th filter</td>
<td>randomised controlled trial or randomised control trial or randomised controlled study or randomised clinical trial or controlled clinical trial or controlled trial or random allocation or single blind or single blind method or single blind trial or double blind method or double-blind method or clinical trial* or quasi-experimental study or placebo-controlled trial or placebo control or intervention study or follow-up stud*</td>
<td>115882</td>
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<td>7</td>
<td>10/12/2014</td>
<td>combined</td>
<td>#3 and #6 and #11 and #14</td>
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<td></td>
</tr>
<tr>
<td>8</td>
<td>10/12/2014</td>
<td></td>
<td>allerg* or atopy or dermatitis or eczema or asthma or wheez* or food hypersensitivity or food allergy or food intolerance or non IgE or non-IgE or mediated allerg* or hypersensitivity or rhinitis</td>
<td>15654</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>10/12/2014</td>
<td>MeSH descriptor: [Hypersensitivity] explode all trees</td>
<td>#8 or #9</td>
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<tr>
<td>10</td>
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<td>#7 or #10</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>10/12/2014</td>
<td></td>
<td></td>
<td>652339</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>10/12/2014</td>
<td>MeSH descriptor: [Randomized Controlled Trial] explode all trees</td>
<td>#12 or #13</td>
<td>139</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>10/12/2014</td>
<td></td>
<td></td>
<td>652339</td>
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<tr>
<td>15</td>
<td>10/12/2014</td>
<td></td>
<td></td>
<td>1465</td>
<td>In Cochrane Reviews (reviews and protocols, other reviews and trials), limited to trials only yields 255 hits</td>
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Search Log-SCOPUS (From inception-January 2015) (appendix 3.2)

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<th>Search No.</th>
<th>Date of search</th>
<th>Concepts</th>
<th>Terms used</th>
<th>Hits</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15/12/2014</td>
<td>1st filter</td>
<td>(TITLE-ABS-KEY(pregnant*) OR TITLE-ABS-KEY(antenatal or ante-natal or ante-natal) OR TITLE-ABS-KEY(prenatal or pre-natal or pre natal) OR TITLE-ABS-KEY(mother or maternal))</td>
<td>1,151,775</td>
<td>Scopus does not use a controlled vocabulary, like MeSH to search</td>
</tr>
<tr>
<td>2</td>
<td>15/12/2014</td>
<td>2nd filter</td>
<td>(TITLE-ABS-KEY(diet*) OR TITLE-ABS-KEY(food or consumption or intake or mineral) OR TITLE-ABS-KEY(nutrition or nutrient) OR TITLE-ABS-KEY(milk or egg or peanut or fruit or vegetable) OR TITLE-ABS-KEY(vitamin* or fatty acid* or supplement*) OR TITLE-ABS-KEY(probiotic* or prebiotic*) OR TITLE-ABS-KEY(folic acid))</td>
<td>3,718,859</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>15/12/2014</td>
<td>3rd filter</td>
<td>(TITLE-ABS-KEY (non-communicable disease*) OR TITLE-ABS-KEY (non communicable disease*) OR TITLE-ABS-KEY (noncommunicable disease*))</td>
<td>7,196</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>15/12/2014</td>
<td></td>
<td>(TITLE-ABS-KEY (allerg*) OR TITLE-ABS-KEY (atopy) OR TITLE-ABS-KEY (dermatitis) OR TITLE-ABS-KEY (eczema) OR TITLE-ABS-KEY (asthma) OR TITLE-ABS-KEY (wheez*) OR TITLE-ABS-KEY (food hypersensitivity) OR TITLE-ABS-KEY (food allergy) OR TITLE-ABS-KEY (food intolerance) OR TITLE-ABS-KEY (non ige) OR TITLE-ABS-KEY (non ige OR mediated allerg*) OR TITLE-ABS-KEY (hypersensitivity) OR TITLE-ABS-KEY (rhinitis))</td>
<td>593,466</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>15/12/2014</td>
<td>#3 or #4</td>
<td></td>
<td>600,386</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>15/12/2014</td>
<td>4th filter</td>
<td>(TITLE-ABS-KEY (randomised controlled trial) OR TITLE-ABS-KEY (randomised control trial) OR TITLE-ABS-KEY (randomised controlled study) OR TITLE-ABS-KEY (randomised clinical trial) OR TITLE-ABS-KEY (controlled clinical trial) OR TITLE-ABS-KEY (controlled trial) OR TITLE-ABS-KEY (random allocation) OR TITLE-ABS-KEY (single blind) OR TITLE-ABS-KEY (single blind method) OR TITLE-ABS-KEY (single blind trial) OR TITLE-ABS-KEY (double blind method) OR TITLE-ABS-KEY (double-blind method) OR TITLE-ABS-KEY (clinical trial*) OR TITLE-ABS-KEY (quasi-experimental study) OR TITLE-ABS-KEY (placebo-controlled trial) OR TITLE-ABS-KEY (placebo control) OR TITLE-ABS-KEY (intervention study) OR TITLE-ABS-KEY (follow-up)</td>
<td>2,636,519</td>
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</tr>
<tr>
<td>7</td>
<td>15/12/2014</td>
<td>combined</td>
<td>#1 and #2 and #5 and #6</td>
<td>1,364</td>
<td>Limited to original studies (n=796)</td>
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374
### Search Log - Other databases (appendix 3.2)

<table>
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<th>Concepts</th>
<th>Terms used</th>
<th>Hits</th>
<th>Comments</th>
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</thead>
<tbody>
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<td>1</td>
<td>10/01/2015</td>
<td>Web of Science</td>
<td>Primary prevention of atopic disease by perinatal dietary interventions</td>
<td>3</td>
<td>One is a published book, might need further attention</td>
</tr>
<tr>
<td>2</td>
<td>10/01/2015</td>
<td>ETHoS</td>
<td>Prevention of atopic disease by maternal dietary interventions in pregnancy</td>
<td>16</td>
<td>all results are also identified in main databases’ search</td>
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<tr>
<td>3</td>
<td>10/01/2015</td>
<td>ETHoS</td>
<td>Prevention of allergic disease by maternal dietary interventions</td>
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<td>4</td>
<td>10/01/2015</td>
<td>Clinicaltrials.gov</td>
<td>Prevention of allergic disease by maternal interventions in pregnancy</td>
<td>12</td>
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</tr>
<tr>
<td>5</td>
<td>10/01/2015</td>
<td></td>
<td>Primary prevention of atopic disease by perinatal interventions</td>
<td>1</td>
<td>Identified in other as well as main data bases</td>
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<tr>
<td></td>
<td>10/01/2015</td>
<td></td>
<td>Prevention of atopic diseases by maternal nutritional interventions in pregnancy</td>
<td>3</td>
<td>Identified in other as well as main data bases</td>
</tr>
<tr>
<td>6</td>
<td>11/01/2015 &amp;</td>
<td>ICTRP**</td>
<td>Prevention of allergic disease (in the title) or pregnancy (in the condition) or nutrition interventions (in the intervention)**</td>
<td>2182</td>
<td>2 relevant study, also found in main data bases</td>
</tr>
<tr>
<td></td>
<td>12/01/2015</td>
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<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Short phrases were used using different synonyms and for each database, the phrases that have yielded any results are shown.

**WHO International Clinical Trials Registry Platform

***Also, the list of trials by health topic, from the WHO above-mentioned platform, was looked into and trials’ titles in some health topics were checked as follows:

a. Child Health (99)
b. Food Safety (3)
c. Food Insecurity (5)
d. Maternal Health (9)
e. Women’s Health (49)
### Study eligibility form-Allergic outcomes

<table>
<thead>
<tr>
<th>PICOS</th>
<th>Yes</th>
<th>Unclear</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Participants</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Are the participants classed as pregnant women, from general population, and their offspring?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Type of study</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Is the study one of the following designs during pregnancy or continued after pregnancy, either in mother or infant or both?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Randomised controlled trial</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cluster randomised controlled trial</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quasi-randomised controlled trial</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Types of interventions</strong> (the intervention could be a combination of the followings)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Food-based dietary advice (promoting a healthy diet) or nutrient intervention</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multivitamins, supplementation and minerals</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fatty acid supplement(s)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre/Probiotic supplement(s)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Outcomes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do the outcome measure allergy, on its own as a general term, or any allergy related outcome(s) i.e. dermatitis, wheeze, asthma, rhinitis?</td>
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<td></td>
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</tr>
<tr>
<td>Are the outcome measures validated?</td>
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### Appendix 3.4-Characteristics of the excluded studies-Allergic outcomes

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<td><strong>Probiotics</strong></td>
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<tr>
<td>Aa 2010</td>
<td>Synbiotics as intervention in infants with AD</td>
</tr>
<tr>
<td>Berni 2011</td>
<td>Post-natal intervention (in infants)</td>
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<tr>
<td>Brouwer 2006</td>
<td>Post-natal intervention (in infants)</td>
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<tr>
<td>Cabana 2007</td>
<td>Post-natal intervention (in infants)</td>
</tr>
<tr>
<td>Chen 2010</td>
<td>Intervention in young children</td>
</tr>
<tr>
<td>Chernyshove 2009</td>
<td>Post-natal intervention (in infants with AD)</td>
</tr>
<tr>
<td>Ciprandi 2005</td>
<td>Post-natal intervention (in infants with allergic rhinitis)</td>
</tr>
<tr>
<td>Cukrowska 2010</td>
<td>Intervention in children with food allergy</td>
</tr>
<tr>
<td>Drago 2011</td>
<td>Intervention in adults with AD</td>
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<tr>
<td>Farid 2011</td>
<td>Synbiotics as intervention in children with AD</td>
</tr>
<tr>
<td>Folster-Holst 2006</td>
<td>Post-natal intervention (in infants with AD)</td>
</tr>
<tr>
<td>Giovannini 2007</td>
<td>Intervention in pre-school children</td>
</tr>
<tr>
<td>Gobel 2010</td>
<td>Intervention in children with AD</td>
</tr>
<tr>
<td>Gore 2012</td>
<td>Post-natal intervention</td>
</tr>
<tr>
<td>Grasimov 2010</td>
<td>Intervention in school children</td>
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<tr>
<td>Gruber 2007</td>
<td>Post-natal intervention (in infants)</td>
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<tr>
<td>Gutkowski 2010</td>
<td>Intervention in children with asthma</td>
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<td>Han 2012</td>
<td>Post-natal intervention (in infants)</td>
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<td>Helin 2002</td>
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<td>Isolauri 2000</td>
<td>Post-natal intervention (in infants)</td>
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<tr>
<td>Kankaanpää 2002</td>
<td>Post-natal intervention (in infants with AD)</td>
</tr>
<tr>
<td>Kobuta 2014</td>
<td>Post-natal intervention (in mothers)</td>
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<tr>
<td>Moro 2006</td>
<td>Post-natal intervention (in infants)</td>
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<tr>
<td>Muraro 2012</td>
<td>Post-natal intervention (in infants)</td>
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<tr>
<td>Prescott 2005</td>
<td>Pre-clinical outcomes reported</td>
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<tr>
<td>Rautava 2006</td>
<td>Post-natal intervention (in mothers)</td>
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<td>Rose 2010</td>
<td>Post-natal intervention (in infants)</td>
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<td>Taylor 2006</td>
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<td>Torii 2011</td>
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<td>Viljanen 2005</td>
<td>Post-natal intervention (in infants with AD)</td>
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<tr>
<td>West 2009</td>
<td>Post-natal intervention (in infants)</td>
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<td>West 2012</td>
<td>Pre-clinical outcomes reported</td>
</tr>
<tr>
<td>Weston 2005</td>
<td>Post-natal intervention (in infants)</td>
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<td>Woo 2010</td>
<td>Intervention in children with AD syndrome</td>
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<tr>
<td>Yesilove 2012</td>
<td>Post-natal intervention (in infants with AD)</td>
</tr>
<tr>
<td><strong>Fatty acids</strong></td>
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</tr>
<tr>
<td>Bergmann 2008</td>
<td>Reported Growth outcomes in infants</td>
</tr>
<tr>
<td>Carlson 2013</td>
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<td>Colombo 2004</td>
<td>Reported growth outcomes in infants</td>
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<td>Courville 2011</td>
<td>Reported growth outcomes at birth</td>
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<td>Dotterud 2013</td>
<td>Non-randomised multifaceted intervention</td>
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<td>Garnot 2011</td>
<td>Reported growth outcomes in infants</td>
</tr>
<tr>
<td>Hauner 2009</td>
<td>Reported growth outcomes in infants</td>
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<td>Helland 2001</td>
<td>Reported growth outcomes in infants</td>
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<tr>
<td>Innin 2007</td>
<td>Reported growth outcomes in infants</td>
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<tr>
<td>Judge 2007</td>
<td>Reported growth outcomes in infants</td>
</tr>
<tr>
<td>Karlsson 2010</td>
<td>Reported growth outcomes in infants</td>
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<tr>
<td>Knudsen 2006</td>
<td>Reported maternal outcome at pregnancy</td>
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<tr>
<td>Martin-Alvarez 2012</td>
<td>Published as an abstract and reported oxidative stress status in infants</td>
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<tr>
<td>Mihralahi 2001</td>
<td>Postnatal diet intervention in infants</td>
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<tr>
<td>Pena-Quintana 2011</td>
<td>Published as an abstract and reported blood DHA concentration</td>
</tr>
<tr>
<td>Romero 2013</td>
<td>Pre-clinical outcomes (genetic programming for allergy in infants)</td>
</tr>
<tr>
<td>Van Gool 2003</td>
<td>Post-natal intervention</td>
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<tr>
<td><strong>Food avoidance</strong></td>
<td></td>
</tr>
</tbody>
</table>
Studies that were excluded at the final stage of screening are presented and also one publication, mainly a recent work for each original study is cited.

**Probiotic studies**

- Cabana MD, McKean M., Wong CC, Caughex AB. Examining the hygiene hypothesis: the Trial of Infant Probiotic Supplementation. Paediatric & Perinatal


Isolauri E, Arvola T, Sutas Y, Moilanen E, Salminen S. Probiotics in the management of atopic eczema. Clinical and Experimental Allergy 2000; 30:1604-10


**Fatty acid studies**


• Karlsson T, Birberg-Thornberg U, Duchen K, Gustafsson PA. LC-PUFA supplemented to mothers during pregnancy and breast-feeding improves cognitive performance in their children four years later - an RCT study. 9th Congress of the ISSFAL; 29 May-2 June 2010; Maastricht, The Netherlands 2010:113.

• Knudsen VK, Hansen HS, Osterdal ML, Mikkelsen TB, Mu H, Olsen SF. Fish oil in various doses or flax oil in pregnancy and timing of spontaneous delivery: a randomised controlled trial. BJOG: an international journal of obstetrics and gynaecology 2006; 113(5): 536–43.


• Mihrshahi S, Peat JK, Marks GB, Mellis CM, Tovey ER, Webb K, Britton WJ, Leeder SR. Eighteen-month outcomes of house dust mite avoidance and dietary fatty acid modification in the Childhood Asthma Prevention Study (CAPS). JACI 2003; 111: 612-168.


**Food avoidance studies**


• Sewell DA, Hammersley VS, Devereux G. Investigating the effectiveness of the Mediterranean diet in pregnant women for the primary prevention of asthma and allergy in high-risk infants: protocol for a pilot randomised controlled trial. Trials 2013; 14, 173.


**Vitamin studies**


Papers published after the search strategy for these systematic reviews were updated on January 2016 (not included in the current review)

<table>
<thead>
<tr>
<th>Study</th>
<th>Intervention and Outcome reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hansen 2016*</td>
<td>Fatty acid, Asthma and allergic respiratory disease at 18-19 years</td>
</tr>
<tr>
<td>Bisgaard 2017</td>
<td>Fatty acid, Asthma/persistent wheeze at 3 years</td>
</tr>
</tbody>
</table>

*updated follow-up (18 years old) of the study by Olsen 2008

Appendix 3.5. Data extraction tool for allergic outcomes

- Screening outcome
  Exclude (Non-Randomised)
  Study Protocol
  Linked Record - Do not obtain data
  Includes abstracts and later follow-up studies, provided that the later follow-up
  has reported the same outcome(s) for the same sample at the earlier report

- Study Details
  Country
  Recruitment Period
  Please record the months/years that the recruitment is done
  Setting
  Where the sample are taken e.g. clinics, hospital, multicentre. Please record
  all reported information (provide page numbers for quotes)
  Informed consent
  If not stated/unclear, please state as "NOT REPORTED/UNCLEAR"
  Ethical Approval
  If stated, please record the relevant information and tick the box.
  If not stated/unclear, please state as "NOT REPORTED/UNCLEAR"
  Source of Funding
  Please record, if stated. If not, please state “not reported”.

- Trial Type
  PC-RCT
  Randomised Controlled Trial - Placebo Controlled
  Please provide brief description of study design.
  RCT
  Randomised Controlled Trial
  Please provide brief description of study design.
  CR
  Cluster Randomised, please provide brief description of study design.
  Quasi Experimental
  Please provide brief description of study design.
  No. & name of Study Groups/Arms
  Please state the number and name of allocated treatment arms i.e. one
  treatment group vs. one placebo group, more than one treatment with/without
  placebo
  Please record all the relevant information briefly
  ITT
  Intention-to-treat analysis
  Yes
  No
  Not mentioned

- Study Sample
  Matched baseline characteristics
  Yes
  No
  Women's age
  If there is any information about women's age, either stated as an inclusion
  criteria or other presented information, please record the age limits
  If possible, split the age data into target group, at the study start, and actual
  reported age in results
  Exclusion criteria
  Yes
  No
  FH of Atopy
  Subjects with a family history of Atopy are selected for the study
Unselected sample
Subjects are taken from an unselected population.
No. of participants at randomisation
Please record the number of PREGNANT WOMEN at the time of randomisation, where recruitment occurred prenatally.
No. at F-U
No. of subjects at the end of follow-up in both intervention and control groups.
Missing Participants
Please record the reasons missing numbers at birth e.g. number of infants eligible at birth.
Reasons Missing
Time Points Measured
Earlier follow-up(s) reported time points
Infant's age at last F-U
Please record the reported age of infants at their last follow-up

- Intervention
  Probiotic Type (Organism)
  Please tick all relevant boxes
    Any Pro
    LGG
    Lactobacillus GG
    LPR+BL999
    ST11 and BL999
    Bifido animalis
    L rhamnosus
    L reuteri
    Mixed Pro
    Added Prebiotic
    If applicable, please record all the relevant information i.e. type, dosage, in mother, infant or both
    Daily Dosage
    Please record the total daily dosage taken in mother, infant or both, whichever is applicable with units

    If more than one type is taken, please record the total daily dosage for all with units
    Mode of Int. Delivery in Infancy
    Who has delivered the intervention and how e.g. mothers and oral
    Mode of Int. delivery during Pregnancy
    e.g. oral use

  FA Type
  Fatty Acid type, please tick all relevant boxes
    Any FA
    N-3 PUFA
    DHA
    Docosahexaenoic Acid
    Fish oil
    Omega-3 PUFA
    Salmon Portions
    SIPS (Salmon In Pregnancy Study)
    Blackcurrant Seed Oil
    Daily Dosage
    Please record the total daily dosage taken in mother, infant or both, whichever is applicable with units.
    If more than one type is taken, please record the total daily dosage for all with units
    Mode of Int. delivery during Pregnancy
Mode of Int. delivery during Infancy

**FI Type**

Food intervention type, please tick all the relevant boxes

- Any FI
- All cow's milk, egg, and peanut products
- Dairy Product & Egg
- All milk and dairy products
- Maternal antigen avoidance
- Continued avoidance in lactation period

Please record if the avoidance is continued after pregnancy, in breast feeding mothers and for how long

- Yes
- No

Mode of intervention delivery in pregnancy

**Vitamin/Suppl. Type**

- Any Vit
- Vit C
- Vit C & E
- Vit D (cholecalciferol)
- Vit D (ergocalciferol)
- Combined Vit D

Daily Dosage

Please record the total daily taken dosage, in mother, infant or both, whichever is applicable with units.

If more than on type is taken, please record the total daily dosage for all with units

Mode of Int. delivery during Pregnancy

Mode of Int. delivery in Infancy

Comparisons

- Placebo
- No treatment
- High diet
- Standard diet

When Int. has been applied?

- Pregnancy alone
- Pregnancy & after delivery-In mothers
- In Pregnancy & after delivery in Mothers & Infants
- Pregnant women & after birth in Infants

Timing in Pregnancy

Please record intake of intervention FROM/UNTIL in pregnant women e.g. 12-40 gestation week.

Duration of Int. in Pregnancy

Please record the total duration of intake, within pregnancy e.g. 6 months

Intake IN MOTHERS after Birth

If the intervention is continued after birth in mothers, please record the intake FROM/UNTIL for that period of time e.g. 6months after birth

Total Duration in Women

Please record the TOTAL duration of intake in MONTHS, including after birth, if continued e.g. 7 months (36 gestation wks. + 6 months after birth)

Timing in Infancy

If applicable, please record the intake of intervention FROM/UNTIL in infants e.g. 6 months

Duration in Infancy

If applicable, please record the total duration of intake in infants after birth, in MONTHS

Total duration of Int.
Feeding Restrictions
If any feeding restrictions are stated in the study i.e. Breast feeding after birth,
Formula feeding (quote the page number)
Type of delivery
Please state if not reported
   Caesarean
   Vaginal
   Instrumental
Side Effects
   Yes
   No
   N/A-NM
   Not Applicable OR Not Mentioned
• Outcomes
  The defined and measured endpoint(s) in the study
  Allergic disease(s)
  If the outcome reported as a general term, as allergic diseases please record
  that and quote the page number/table number.
  Wheeze
  Eczema
  Asthma
  SPT (any positive)
  SPT (egg)
  SPT (peanut)
  SPT (HDM)
  SPT (cows milk)
  SPT (CAT)
  SPT (Cod)
  SPT (grass)
  SPT (milk)
  SPT (food)
  SPT (wheat)
  SPT (Dog)
  Cashew nut sensitization
  Sesame seed sensitization
  Specific IgE
  Food Allergy
  Anaphylaxis
  Angioedema
  Olive tree sensitization
  Dermatophagoides pteronyssinus sensitization
  Dermatophagoides farinae sensitization
  Sensitisation
  AD
  Atopic Dermatitis
  Rhinitis
  Allergic rhinoconjunctivitis
  Respiratory allergy
  Urticaria
  Allergy Atopy
  If the outcome is reported as Allergy atopy, please specify i.e. asthma, allergic
  rhinoconjunctivitis (ARC), allergic urticaria and eczema
  Bronchial Obstruction
  Cough
  Chronic cough
  Chest infection
  Breathing Difficulty
  Pneumonia/bronchiolitis
Phlegm & Nasal Discharge
Fever
Atopic Eczema Dermatitis Syndrome
Sneezing and/or snuffling
Atopy
URTI
*upper respiratory tract infection*
LRTI
*lower respiratory tract infection*
SCORAD
SCORAD >= 25
SCORAD 0
SCORAD 1-25
SCORAD 25-50
SCORAD > 50
SPT (aeroallergen)
SPT (Inhalation allergens)
SPT (Birch)
SPT (Alder)
SPT (Derp 1)
Any IgE
Atopic sensitized
Received topical steroid preparation
Inhaled bronchodilator or steroid
Itchy skin
Dry skin
*Alternaria tenuis* sensitization

- **Diagnosis Method**

  *Please tick all that apply*

  ISAAC
  *Provide a brief description how the questionnaires have been completed e.g. clinicians, nurse, parents*
  Clinician diagnosed
  SPT
  *Skin Prick Test*
  *If the test is conducted vs. any specific allergen, e.g. egg-peanut-milk, please record the details including the mean diameter of the wheal e.g. >= 3 mm*
  Self report
  *Please record the mode of report e.g. parents*
  SCORAD
  BCSS
  *basic clinical scoring system*
  Research nurses/staff
  FEno
  *Fractional Exhaled nitric oxide*
  FEV
  *forced expiratory volume*
  Spirometry tests
  *e.g. Forced Expiratory Volume (FEV), Functional Vital Capacity (FVC), positive reversibility tests*
  Clinical history
  Physical examination
  IgE
  *Measurement of IgE in cord blood e.g. cut off points >0.35 KU/L*
  F-U Questionnaires
  Nottingham Eczema Severity Score (NESS)
  UK Eczema Working Party criteria
Asthma predictive index
U.K. Working Party’s diagnostic criteria for AD
Primary health care records
Cow's milk challenges
eNO
*Exhaled nitric oxide*
IOS
*Impulse oscillometry*
ARIA guidelines
Williams UK Working Party’s criteria
the British Medical Research Council questionnaire
the European Community Respiratory Health Survey
Molecular analysis of faecal microbiota
Hanifin
SASSAD
*the Six Area Six Sign in Atopic dermatitis (SASSAD) score*
Open food challenge
Blood sample
Saliva
NPR
*National Patient Registry*

- **Outcome Classifications**
  - FH of Atopy
  - Unselected Sample
  - At risk for pre-eclampsia
  - Probiotics
  - Fatty Acids
  - Food Intervention
  - Vitamins
Appendix 3.6. Risk of bias judgement (pro/prebiotic trials for prevention of allergic outcomes)

<table>
<thead>
<tr>
<th>Study author</th>
<th>Random Sequence Generation</th>
<th>Allocation Concealment</th>
<th>Double Blinding</th>
<th>Blinding of Outcome Assessment</th>
<th>Incomplete Outcome Data</th>
<th>Selective Outcome Reporting</th>
<th>Other Sources of Bias</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kalliomäki (2003)</td>
<td>• Low Mothers were randomly assigned to the study arms using computer generated random sequence</td>
<td>• Low Treatment codes were kept by the supplier until data had been collected and analysed</td>
<td>• Low LGG and placebo capsules and contents looked, smelled, and tasted identical. Also, treatment codes were kept by the supplier until data had been collected and analysed</td>
<td>• Low The diagnosis of atopic disease, made by a researcher (TP) who was unaware of the treatment allocation.</td>
<td>• Low Low lost to follow-up and the clinical outcomes are reported for all that followed-up</td>
<td>• Low all pre-defined (as reported in the methods) outcomes are reported</td>
<td>• Unclear No report as to whether the any commercial probiotics have been consumed after the 2yrs F-U.</td>
</tr>
<tr>
<td>Kalliomäki (2007)</td>
<td>• Low Same as Kalliomäki 2003</td>
<td>• Low Same as Kalliomäki 2003</td>
<td>• Unclear No clear information whether the staff were un-blinded after the 2yrs follow-up</td>
<td>• Low Diagnosis of eczema was made blindly on the basis of both a questionnaire and clinical examination</td>
<td>• High The reasons for attrition at 7yrs follow-up not specified and also SPT conducted in a sub-sample of 109 children at 7yrs.</td>
<td>• Low Fewer outcomes reported compared to earlier report i.e. food allergy, specific SPTs</td>
<td>• Unclear No report as to whether any commercial probiotics have been consumed later on.</td>
</tr>
<tr>
<td>Huurre (2008)</td>
<td>• Unclear Method of randomisation not mentioned</td>
<td>• Unclear - Does not state anything about allocation concealment in either paper</td>
<td>• Unclear No clear information (either in 2006 or 2008 papers), just says women were randomised in a double-blind manner</td>
<td>• Low All infants were clinically examined in blindly</td>
<td>• High the SPT and pre-clinical results reported for different sub-samples</td>
<td>• Low The pre-defined data including infant sensitisation is reported</td>
<td>• High No information on the consumption of probiotics after delivery</td>
</tr>
<tr>
<td>Kopp (2008)</td>
<td>• Low Randomization was performed in blocks of 4 according to a computerized randomisation list</td>
<td>• Unclear No information about how allocation happened</td>
<td>• Unclear No information about blinding of research team. LGG and placebo were matched for appearance, taste, smell and packing</td>
<td>• Low Physicians were unaware of the contents of the capsules until the end of the study</td>
<td>• Low Reasons of missing are similar between the groups</td>
<td>• Low All the pre-defined primary &amp; secondary outcomes are reported.</td>
<td>• Low None identified</td>
</tr>
<tr>
<td>author</td>
<td>Random Sequence Generation</td>
<td>Allocation Concealment</td>
<td>Double Blinding</td>
<td>Blinding of Outcome Assessment</td>
<td>Incomplete Outcome Data</td>
<td>Selective Outcome Reporting</td>
<td>Other Sources of Bias</td>
</tr>
<tr>
<td>---------------</td>
<td>----------------------------</td>
<td>------------------------</td>
<td>-----------------</td>
<td>--------------------------------</td>
<td>------------------------</td>
<td>---------------------------</td>
<td>----------------------</td>
</tr>
<tr>
<td>Niers (2009)</td>
<td>• <strong>Unclear</strong> - Does not state how randomisation was conducted (apart from stating that it was block randomisation)</td>
<td>• Unclear</td>
<td>No information provided about allocation concealment</td>
<td>• Unclear</td>
<td>No information on allocation concealment, however both supplements were identical.</td>
<td>• <strong>Low</strong> Physicians were blinded with respect to group allocation until all children were seen at the age of 2yrs</td>
<td>• <strong>Low</strong> The pre-defined outcomes (as reported in the methods) are reported in detail</td>
</tr>
<tr>
<td>Kuitunen (2009)</td>
<td>• <strong>Low</strong> Computer-generated block randomization</td>
<td>• Low</td>
<td>Throughout the study, the randomisation code was kept by the database consultant and revealed to the statistician only</td>
<td>• <strong>Low</strong></td>
<td>The randomisation code was kept by the database consultant and revealed to the statistician only. The capsules and syrups, looked, smelled, and tasted identical.</td>
<td>• <strong>Unclear</strong> No statement as to whether the paediatrician was blinded to the treatment allocation at 5yrs F-U</td>
<td>• <strong>Low</strong> All pre-defined primary &amp; secondary outcomes are reported</td>
</tr>
<tr>
<td>Kim (2010)</td>
<td>• <strong>Low</strong> Allocation to either probiotics or placebo was carried out using a computerised generated randomisation</td>
<td>• Low</td>
<td>Treatment of either probiotics or placebo was allocated by trials coordinator without detailed knowledge of the clinical history</td>
<td>• <strong>Low</strong></td>
<td>Probiotic and placebo sachets contents looked, smelled, and tasted identical, plus blinding of trial coordinators</td>
<td>• <strong>Low</strong> The paediatric allergist remained unaware of the actual treatment</td>
<td>• <strong>Low</strong> The pre-defined outcomes (as reported in the methods) are reported</td>
</tr>
<tr>
<td>Study author</td>
<td>Random Sequence Generation</td>
<td>Allocation Concealment</td>
<td>Double Blinding</td>
<td>Blinding of Outcome Assessment</td>
<td>Incomplete Outcome Data</td>
<td>Selective Outcome Reporting</td>
<td>Other Sources of Bias</td>
</tr>
<tr>
<td>Dotterud (2010)</td>
<td>• <strong>Low</strong> Through a computer-generated</td>
<td>• Low</td>
<td>Participants &amp; investigators were</td>
<td>• <strong>Low</strong></td>
<td>The computer-generated randomisation list was</td>
<td>• Unclear Reasons for discontinued</td>
<td>• Low None was identified</td>
</tr>
</tbody>
</table>
randomisation list without restrictions, the Department of Applied Clinical Research at the Norwegian University of Science and Technology randomly assigned the participants. The probiotic & placebo products, in equal tastes and neutral packaging, distributed according to the randomisation list by a Norwegian company (Tine BA). revealed to the researchers once all of the participants had completed the end-point examinations, including the SPTs and specific IgE analyses at 2yrs of age. randomisation list was revealed to the researchers once all of the participants had completed the end-point examinations, at 2yrs of age.

<table>
<thead>
<tr>
<th>Study author</th>
<th>Random Sequence Generation</th>
<th>Allocation Concealment</th>
<th>Double Blinding</th>
<th>Blinding of Outcome Assessment</th>
<th>Incomplete Outcome Data</th>
<th>Selective Outcome Reporting</th>
<th>Other Sources of Bias</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boyle (2011)</td>
<td>Low Using a computer generated randomisation list stratified by number of parents affected by allergic disease ('2' versus '1 or 0').</td>
<td>Low Treatment was allocated by a hospital pharmacist at enrolment according to the order in which subjects were recruited</td>
<td>Low Participants, clinical trial and laboratory staff were blinded to treatment allocations throughout the study</td>
<td>Low Participants, clinical trial and laboratory staff were blinded to treatment allocations throughout the study</td>
<td>Low Reasons of missing are explained at each stage of F-U.</td>
<td>Low All pre-defined outcomes (as reported in the methods) are reported</td>
<td>Low None identified</td>
</tr>
<tr>
<td>Rautava (2012)</td>
<td>Low The allocation was carried out using a computer-generated list independently from the investigators</td>
<td>Low All investigations were performed in a double-blind fashion, and the allocation code was revealed after all the infants had completed the F-U and the data had been finalised.</td>
<td>Low All investigations were performed in a double-blind fashion, and the allocation code was revealed after all the infants had completed the F-U and the data had been finalised.</td>
<td>Low The rate of discontinuing the study or lost to follow-up was similar in the 3 study groups.</td>
<td>Low The pre-defined primary outcome measures (as reported in the methods) reported</td>
<td>Low None identified</td>
<td>Low None identified</td>
</tr>
</tbody>
</table>

Study author: Boyle (2011) and Rautava (2012)
### Ou (2012)

- **Random Sequence Generation**: Unclear
- **Allocation Concealment**: Low
  - Treatment codes were kept by the supplier until all data had been collected and analysed.
- **Double Blinding**: Low
  - The treatment codes were kept by the supplier until all data was collected and analysed.
- **Blinding of Outcome Assessment**: Low
  - The group allocation concealed until completion of data analysis.
- **Incomplete Outcome Data**: Unclear
  - Reasons for lost to follow-up in study arms not specified (Figure 1)
- **Selective Outcome Reporting**: Low
  - All pre-defined outcomes (as reported in the methods) reported including Cumulative prevalence
- **Other Sources of Bias**: Unclear
  - No information whether women have continued consumption of probiotics after the termination of intervention

### Abrahamsson (2013)

- **Random Sequence Generation**: Unclear
- **Allocation Concealment**: Low
  - Each centre was provided an allocation list with unique ID No. for each subject. Prior to randomisation, each study product bottle was labelled with the unique ID No. and randomly mixed by an independent contract manufacturer.
- **Double Blinding**: High
  - The study was conducted in a double blind fashion until all infants had completed the 2yrs follow-up.
- **Blinding of Outcome Assessment**: High
  - There was high loss to follow-up, but equally spread across both groups. However, reasons for being lost to follow-up are not reported.
- **Incomplete Outcome Data**: Low
  - All pre-defined outcomes (as reported in the methods) are reported
- **Selective Outcome Reporting**: High
  - Infants in the placebo group did not receive the supplement after birth. Also, At 7y of age, 19% in the L. reuteri and 26% in placebo group reported to have taken any probiotic strain during the last month (p=0.30).

### Wickens (2013)

- **Random Sequence Generation**: Low
  - Randomisation was stratified by study centre and performed in blocks of 15 according to a computer-generated randomisation list.
- **Allocation Concealment**: Low
  - Randomisation and allocation of supplements were performed by a clinical trials pharmacist with no contact with the participants.
- **Double Blinding**: High
  - After the 2-year follow-up parents were not blind to study group, and were subsequently asked to report subjectively on eczematous symptoms.
- **Blinding of Outcome Assessment**: Low
  - Study nurses remained blinded to participant study group through-out.
- **Incomplete Outcome Data**: High
  - Imputed analysis is used for some of the reported outcomes; also the reasons for lost to F-U are not specified.
- **Selective Outcome Reporting**: Low
  - All pre-defined outcomes (as reported in the methods) are reported
- **Other Sources of Bias**: Low
  - Infants excluded if exposed to commercially available non-study probiotics, either directly or through breast milk during the course of the study
<table>
<thead>
<tr>
<th>Study</th>
<th>Allocation Method</th>
<th>Allocation Concealment</th>
<th>Outcome Reporting</th>
<th>Participation</th>
<th>Baseline Characteristic</th>
<th>Non-identified Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allen (2014)</td>
<td>A computer-generated, random allocation sequence, produced by the independent statistician, without blocks allocated the mother-infant dyad at 36wks of gestation to either the treatment or placebo arm of the study on a 1:1 basis.</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>Non identified</td>
</tr>
<tr>
<td>Gorissen (2014)</td>
<td>It does not say how randomisation was conducted apart from block randomization with a block size of 10 was used.</td>
<td>Unclear</td>
<td>High</td>
<td>Low</td>
<td>Low</td>
<td>No information whether the study participants consumed probiotic products, from the last F-U at 2yrs age</td>
</tr>
<tr>
<td>Simpson (2015)</td>
<td>Through a computer-generated randomisation list without restrictions, the Department of Applied Clinical Research at the</td>
<td>Low</td>
<td>High</td>
<td>High</td>
<td>Low</td>
<td>None identified</td>
</tr>
<tr>
<td>Norwegian University of Science and Technology randomly assigned the participants.</td>
<td>neutral packaging, distributed according to the randomisation list by Tine BA, a Norwegian company.</td>
<td>group</td>
<td>are also reported</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Data extracted from original and companion papers, where applicable*
### Appendix 3.7. Risk of bias judgement (fatty acid trials for prevention of allergic outcomes)*

<table>
<thead>
<tr>
<th>Study author</th>
<th>Random Sequence Generation</th>
<th>Allocation Concealment</th>
<th>Double Blinding</th>
<th>Blinding of Outcome Assessment</th>
<th>Incomplete Outcome Data</th>
<th>Selective Outcome Reporting</th>
<th>Other Sources of Bias</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dunstan (2003a)</td>
<td>• Unclear The groups were block-randomized according to parity, but not mentioned how</td>
<td>• Low Randomisation and allocation of capsules occurred at a different centre separate from the recruitment of participants.</td>
<td>Low The participants, research scientists, and pediatrician remained blinded to the groups for the duration of the study. The capsules in the 2 groups were image-matched</td>
<td>• Low A detailed history and examination by the same pediatrician (SLP) who remained blinded to the intervention</td>
<td>• High A high lost to F-U rate was observed in the Int. arm (12 vs. 3) and the discontinuation rate due to nausea in the Int. group was higher than the control (7 vs. 1)</td>
<td>• Low All the pre-defined outcomes are reported</td>
<td>• Low None identified, however it is worth note that only healthy infants were included in the data analysis, to avoid the confounding effects of prematurity</td>
</tr>
<tr>
<td>Olsen (2008)</td>
<td>• Unclear Although the authors report that they conducted stratified randomisation, they do not report how randomisation itself was conducted</td>
<td>• Low Women who agreed to participate, were allocated by randomisation numbers concealed in sealed envelopes</td>
<td>• High Women would know if they were in the control group, as they would not receive any tablets. This would very much depend on what information was provided to participants. If they are fully informed (i.e. in order to give proper consent) then they should have been told what being part of the control group would involve. Does not report blinding of investigators.</td>
<td>• Low Diagnoses were external to study team by using a unique 10-digit personal identification number with a link from mother to child and vice versa</td>
<td>• Low A few numbers are missing from two study groups which are reported with the reasons missing.</td>
<td>• Low All pre-defined outcomes (as reported in the methods) are reported</td>
<td>• Low None identified</td>
</tr>
<tr>
<td>Study author</td>
<td>Random Sequence Generation</td>
<td>Allocation Concealment</td>
<td>Double Blinding</td>
<td>Blinding of Outcome Assessment</td>
<td>Incomplete Outcome Data</td>
<td>Selective Outcome Reporting</td>
<td>Other Sources of Bias</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------------------------</td>
<td>------------------------</td>
<td>-----------------</td>
<td>-------------------------------</td>
<td>------------------------</td>
<td>--------------------------</td>
<td>----------------------</td>
</tr>
<tr>
<td>Linnamaa (2010)</td>
<td>Low Randomisation was assigned by a random number list</td>
<td>Low The random allocation sequence was concealed until interventions were assigned. Different personnel than randomization carried out recruitment and subsequent contacts with the study subjects.</td>
<td>Low Both oils were similar, applied to mothers &amp; infants. Recruitment &amp; subsequent contacts with the study subjects were carried out by different personnel than randomization</td>
<td>Low The treatment codes were not opened until the study material had been analysed in March 2008.</td>
<td>Low Lost to F-U were similar across the study arms; however a high rate (&gt;50%) was observed mostly due to pregnancy related nausea and poor compliance</td>
<td>Low The pre-defined study endpoints (as reported in the methods) are reported</td>
<td>Low None identified</td>
</tr>
<tr>
<td>Furuhjelm (2011)</td>
<td>Unclear Says block randomisation was performed, but now how</td>
<td>Unclear No information about how women were allocated</td>
<td>Low The mothers, as well as the staff handling clinical and laboratory F-U, were blinded to group allocation. Active and placebo capsules could not be distinguished from each other.</td>
<td>Low The research nurses, the paediatricians &amp; the person performing the laboratory analyses were blinded during the intervention and F-U. Further, all staff members working with the intervention and F-U were blinded throughout the whole study</td>
<td>High Sixteen (23%) of the mothers in the Int. arm and 9 (12%) in the placebo group did not complete the minimum 15wk of supplementation required, i.e. throughout pregnancy. Also 54 (77%) and 65 (87%) of women in the int. and placebo arms were approached respectively at 2yrs F-U.</td>
<td>Low All pre-defined outcomes (as reported in the methods) are reported</td>
<td>Low None identified</td>
</tr>
<tr>
<td>Noakes (2012)</td>
<td>Low The women were allocated to 1 of 2 groups according to a previously</td>
<td>Unclear No reference made to allocation concealment</td>
<td>High Single blind study, researchers responsible for assessing outcome measures (both laboratory and clinical)</td>
<td>Low Researchers responsible for assessing outcome measures (both laboratory and clinical)</td>
<td>High Reasons for lost to F-U not mentioned</td>
<td>Low All pre-defined outcomes (as reported in)</td>
<td>Low None identified</td>
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<tr>
<td>Study author</td>
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<td>Allocation Concealment</td>
<td>Double Blinding</td>
<td>Blinding of Outcome Assessment</td>
<td>Incomplete Outcome Data</td>
<td>Selective Outcome Reporting</td>
<td>Other Sources of Bias</td>
</tr>
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</tr>
<tr>
<td>Palmer (2013)</td>
<td>• Low A computer driven telephone randomisation service according to an independently generated randomisation schedule, with balanced variable sized blocks.</td>
<td>• Low The allocation sequence was held off site and managed by random number allocation</td>
<td>• Low Neither the women nor the research staff were aware of the treatment allocated. All capsules were similar in size, shape and colour.</td>
<td>• Low All staff were blinded to treatment group allocation and had quality assurance reviews every 6 months with one of the investigators.</td>
<td>• Low Reasons for missing in both group are similar and fully reported, imputed analysis with 50 complete data sets were used which appeared reasonable for their data</td>
<td>• Low All predefined outcomes (as reported in the methods) are reported.</td>
<td>• Low None identified</td>
</tr>
<tr>
<td>Escamilla-Nuñez (2014)</td>
<td>• Unclear Says block randomisation to randomly create balanced replication of four treatments, but does not say how</td>
<td>• Low The assignment codes were placed in sealed envelopes at the beginning of the study.</td>
<td>• Low All study participants and members of the study team were blinded to the treatment scheme throughout the intervention period of the study. The placebo capsules were similar in appearance &amp; taste to DHA capsules.</td>
<td>• Low Data were unblended for the analytical study team after the last baby in the study was born and had reached 6 months of age, at which time participants were no longer taking supplements. Since the study is on going for F-U of children, the participants and fieldworkers remain blinded to the treatment allocation.</td>
<td>• Low Reasons missing are explained in Figure 1 and the statistical analysis is performed on 869 as the total available sample of mother-child pairs</td>
<td>• Low All the predefined outcomes (as reported in the methods) are reported.</td>
<td>• Low None identified</td>
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</table>

*Data extracted from original and companion papers, where applicable*
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<th>Random Sequence Generation</th>
<th>Allocation Concealment</th>
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<th>Blinding of Outcome Assessment</th>
<th>Incomplete Outcome Data</th>
<th>Selective Outcome Reporting</th>
<th>Other Sources of Bias</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lilja (1989)</td>
<td>• Unclear</td>
<td>• Unclear</td>
<td>• High</td>
<td>• Low</td>
<td>• Low</td>
<td>• Low</td>
<td>• High</td>
</tr>
<tr>
<td></td>
<td>Says randomly allocated but not how</td>
<td>No clear information about how allocation was conducted</td>
<td>It would not be possible to blind participants to this intervention, they would have known which group they were in.</td>
<td>The physicians who performed the physical examination at 18 months of age were unaware of the mothers’ diet during the trial</td>
<td>There was attrition but reasons for withdrawal are presented and do not appear to be linked to the intervention</td>
<td>The risk of atopic disease has been defined as the main outcome and, based on that the results (as reported in the methods) are presented in detail in tables.</td>
<td>Women were initially selected from 3 cities with four different allocated interventions and these are pooled together in their further reports introduced as high and reduced diets. Also some women in the reduced group have decided, on their own, to continue their diet during breast-feeding. The actual number of participants presented for the outcomes do not also match with the numbers allocated to each group after exclusion (Table 1).</td>
</tr>
<tr>
<td>Fälth-Magnusson (1992)</td>
<td>• Unclear</td>
<td>• Unclear</td>
<td>• High</td>
<td>• Unclear</td>
<td>• High</td>
<td>• Low</td>
<td>• High</td>
</tr>
<tr>
<td></td>
<td>Says mothers randomised, but not how</td>
<td>there is no information about allocation concealment.</td>
<td>it is not possible to blind due to the nature of the intervention.</td>
<td>No information as to whether the specialised nurse or the author who did the physical examinations were blind to allocation groups</td>
<td>22 women switched from D to ND, and 7 from ND to D, without allocating to it. Although both groups were similar at baseline for allergy-related characteristics and it could be assumed that the reasons for switching groups were unrelated to this and/or would have no bearing on the allergy outcomes.</td>
<td>All the symptoms of allergy according to the questionnaires are reported and explained as well as signs at examination, however assessment of allergic disease by authors is presented as a percentage only</td>
<td>Some mothers by their own choice continued to restrict their intake of cow’s milk and egg during the first 6 weeks of lactation. Also, 10 mothers receiving the diet continued a strictly egg and milk-free diet, whereas 57 D and 24 ND mothers took reduced amounts of cow’s milk and egg (at most 2 dl of milk per day and two eggs per week). There was also a significant difference between D and ND group children for smoking behaviour of their parents.</td>
</tr>
<tr>
<td>Study author</td>
<td>Random Sequence Generation</td>
<td>Allocation Concealment</td>
<td>Double Blinding</td>
<td>Blinding of Outcome Assessment</td>
<td>Incomplete Outcome Data</td>
<td>Selective Outcome Reporting</td>
<td>Other Sources of Bias</td>
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<td>--------------------------------</td>
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<td>---------------------</td>
</tr>
<tr>
<td>Zeiger (1992)</td>
<td>Low A computer generated random numbers list was used</td>
<td>Unclear No information about method of allocation is presented</td>
<td>High Although this was attempted at all times did not remain absolute, since occasionally the infant’s group or formula was designated by the mother or noted in the medical chart by a pediatrician.</td>
<td>Unclear Evaluation of outcomes was based on medical record review and physical examination were performed without physician knowledge of the subject’s group, although unmasking occurred due to parental assertion to the primary pediatrician who recorded on rare occasion.</td>
<td>High The trial has a gross loss to F-U, although says similar rates of attrition were observed in both study groups (page 5, results), the number loss-to-follow are high.</td>
<td>Low Pre-defined outcomes (as reported in the methods) are reported in detail</td>
<td>Low Interventions common to both groups: Breastfeeding was recommended for at least 4 to 6 months. Infant vitamin supplementation was left to the discretion of the personal pediatrician. Parents were encouraged, through information exchange (lecture, slide presentation, and brochures), to reduce household aerosollergens and to discontinue smoking. Mean heights and weights at 3 &amp; 4 years were similar in the study groups (data not shown).</td>
</tr>
<tr>
<td>Lovegrove (1994)</td>
<td>High The atopic group was randomly allocated into the prophylaxis group or the control group, but is not reported how.</td>
<td>Unclear whether allocation concealment has occurred is not reported.</td>
<td>High On admittance to hospital the nurses were informed about the maternal dietary restriction and the necessity not to give the babies ‘top-up’ bottle-feeds.</td>
<td>Low A ‘blind’ physical examination, was performed by a paediatrician who was unaware to which group the infants’ mothers were assigned.</td>
<td>Low There is no missing data and a subgroup analysis also conducted excluding the 3 infants in the atopic-diet group who inadvertently received at least 1 feed of commercial infant formula derived from cow’s milk shortly after delivery.</td>
<td>Low All the pre-defined outcomes, pre-clinical and clinical (as reported in the methods) are reported</td>
<td>Unclear The study is generously supported by Cow &amp; Gate, Trowbridge, Wilts and also provided Peptijunior, a hypoallergenic formula that was used as a milk alternative for the mother and infant in their study.</td>
</tr>
</tbody>
</table>

*Data extracted from original and companion papers, where applicable*
### 3.9. Risk of bias judgement (vitamin trials for prevention of allergic outcomes)

<table>
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<tr>
<th>Study author (year)</th>
<th>Random Sequence Generation</th>
<th>Allocation Concealment</th>
<th>Double Blinding</th>
<th>Blinding of Outcome Assessment</th>
<th>Incomplete Outcome Data</th>
<th>Selective Outcome Reporting</th>
<th>Other Sources of Bias</th>
</tr>
</thead>
<tbody>
<tr>
<td>Greenough (2010)</td>
<td>• Unclear The randomisation sequence was blocked—i.e., balanced—but not how randomisation sequence was generated</td>
<td>• Low DHP Investigational Medicinal Products Clinical Trial Supplies (Crickhowell, Powys, Wales, UK) packaged the tablets and capsules sealed in blister strips each with 1 week’s supply, according to the randomisation sequence provided</td>
<td>• High The trial staff or other person involved in the trial were blind to the allocated treatment until after completion of the VIP trial (the 1st phase of the study). There is no information whether mothers were still blind to their allocated treatment as says they were sent the information about the respiratory F-U study</td>
<td>• Low Researchers assessing the respiratory outcome of the infants were blind to the maternal allocation of treatment</td>
<td>• High A high rate of lost to follow-up was observed due to no response/mother declined</td>
<td>• Low Detailed report of all pre-defined outcomes (as reported in the methods)</td>
<td>• High No information whether women have consumed vitamin C &amp; E after birth. Also this study is an unplanned F-U of an RCT especially as the authors are not clear whether mothers were made aware of their allocation at the planned end of the trial. If they were made aware this could have disproportionately influenced which mothers decided to participate in the F-U. Having said that, roughly equal numbers from each group participated in this follow-up.</td>
</tr>
<tr>
<td>Goldring (2013)</td>
<td>• Low A computer generated random number list in blocks of 15,</td>
<td>• Low The researcher gave participants a study number on entry to the trial,</td>
<td>• High Not possible to blind participants or investigators as would know if</td>
<td>• Low Investigators kept blind to original treatment allocation assessed offspring at</td>
<td>• Low Reasons for missing data are reported and well balanced between the groups.</td>
<td>• Low All defined outcomes (as reported in the methods) are</td>
<td>• Low This trial was conducted before national guidance on routinely providing</td>
</tr>
</tbody>
</table>
stratified by 4 ethnic groups in a 1:1:1:1 ratio and treatment was allocated from the hospital pharmacy. They had no treatment, daily tablets or a single bolus.

Sensitivity analysis was also performed, as 22 offspring were not followed up. There was no significant difference between groups. Sensitivity analysis was also performed, as 22 offspring were not followed up. There was no significant difference between groups.

| McEvoy (2014) | Unclear Randomization was stratified according to gestational age at randomization but the method of randomisation was not reported. | Unclear No information on how allocation has happened | Low The investigators, clinicians and patients were unaware of treatment allocation through age 1 yr and analyses of all primary and secondary outcomes. The OHSU research pharmacy dispensed study capsules. | Low Clinical research personnel unaware of treatment assignment administered the respiratory questionnaire17 (pediatric version) to the infant’s primary caretaker when the infant was approximately 12mo old. | Low Number of missing are equal in both study groups (for pre-clinical and clinical measurements at birth & 1yr) | Low None identified |

| Chawes (2016) | Low Women were randomized using a computer-generated list of random numbers, supplied by an external investigator who | Unclear No information how allocation has happened | Low The intervention code was unblinded when the youngest child reached age 3 years or in case of a medical emergency. | Low The study paediatricians, acted as general practitioners for the cohort, were blinded to the intervention. | Low Between 6.3-7% of women are lost to F-U. However 96.5% of pregnant women did not respond to the initial invitation letters to study. Of women responded, | Low None identified |

Women were also received n-3 LCPUFA supplementation during pregnancy and this is not stated how this could have enhanced the effect of Vit D3. The statistical analyses did
had no further involvement in the RCT

66.8% were excluded or declined and lost due to delayed ethical approval of the trial.

not show an interaction with the supplementation effect, univariate & multivariate.

• Low
Randomization was performed by the Data Centre Coordinating (DCC) using a system that automates the random assignment of treatment groups to Study ID No. The randomization scheme employed stratified permuted blocks with randomly varied block sizes of 4 and 6, and one block allocation list per stratum (study site and racial/ethnic group).

• Low
Clinical Centre investigators and staff were blinded to the treatment code. The pill bottles labelled as “Study Drug A” through “Study Drug F” were shipped to the centres and each participant received 2 bottles (containing the standard prenatal 400IU Vit. D, and the other bottle contained the Int. pill containing 4000IU Vit D or a placebo).

• Low
The study protocol says clinical centre investigators and staff were blind to the treatment code. After delivery, the research staff made telephone calls every 3 months inquiring the health and symptoms of infants. The mother and child came in for 3 yearly follow-up visits, during which blood was drawn, skin pigmentation tests were performed, additional questionnaires were administered, and anthropometric measurements of the child were obtained.

• Low
Between 7-8% of participants are missing and reasons are similar across study arms.

• Low
All pre-defined outcomes in protocol are reported

• Low
None identified

1Data extracted from original and companion papers, where applicable
Appendix 4.1. Search strategy (obesity outcomes)

The following four important concepts were identified based on the research question:

- Pregnancy
- Diet
- Obesity (Non-communicable diseases)
- Randomised controlled trial

A variety of keywords and synonyms were identified using the Patient Intervention Comparison Outcome Study (PICOS) approach. A full list of the used keywords for each concept in the databases is presented in the following table.

Within groups of terms, the terms were combined using OR and the groups of terms themselves were then combined in the following manner: #1 AND #2 AND #3 AND #4.

Relevant citations and key authors were identified. To prevent bias, no restriction was placed on the year of publication or language.
### Search Log-MEDLINE (From inception-February 2015) (appendix 4.1)

<table>
<thead>
<tr>
<th>Search No.</th>
<th>Date of search</th>
<th>Concepts</th>
<th>Terms used</th>
<th>Hits</th>
<th>Comments</th>
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<td>pregnan* OR (antenatal OR ante-natal OR “ante natal”) OR (prenatal OR pre-natal OR “pre natal”) OR (mother* OR maternal)</td>
<td>975,607</td>
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<td>(MH &quot;Pregnancy+)&quot;)</td>
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</tr>
<tr>
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<td>diet* OR (food or consumption or intake) OR (mineral or nutrition or nutrient) OR (milk or egg or peanut or fruit or vegetable) OR (vitamin* or fatty acid* or supplement*) OR (probiotic* or prebiotic*) OR folic acid</td>
<td>1,982,174</td>
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<td>1,134,412</td>
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<tr>
<td>13</td>
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<td></td>
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<td>S3 AND S6 AND S11 AND S14</td>
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### Search Log—COCHRANE (From inception-February 2015) (appendix 4.1)

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### Search Log-SCOPUS (From inception-February 2015) (appendix 4.1)

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<td>Scopus does not use a controlled vocabulary, like MeSH to search</td>
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<tr>
<td>6</td>
<td>12/02/2015</td>
<td>4th filter</td>
<td>(TITLE-ABS-KEY (randomised controlled trial) OR TITLE-ABS-KEY (randomised control trial) OR TITLE-ABS-KEY (randomised controlled study) OR TITLE-ABS-KEY (randomised clinical trial) OR TITLE-ABS-KEY (controlled clinical trial) OR TITLE-ABS-KEY (controlled trial) OR TITLE-ABS-KEY (random allocation) OR TITLE-ABS-KEY (single blind) OR TITLE-ABS-KEY (single blind method) OR TITLE-ABS-KEY (single blind trial) OR TITLE-ABS-KEY (double blind method) OR TITLE-ABS-KEY (double-blind method) OR TITLE-ABS-KEY (clinical trial*) OR TITLE-ABS-KEY (quasi-experimental study) OR TITLE-ABS-KEY (placebo-controlled trial) OR TITLE-ABS-KEY (placebo control) OR TITLE-ABS-KEY (intervention study) OR TITLE-ABS-KEY (follow-up)</td>
<td>2,636,519</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>12/02/2015</td>
<td>combined</td>
<td>#1 and #2 and #5 and #6</td>
<td>4,926</td>
<td>Limited to original studies (n=3,636)</td>
</tr>
</tbody>
</table>
### Search Log - Other databases (appendix 4.1)

<table>
<thead>
<tr>
<th>Search No.</th>
<th>Date of search</th>
<th>Concepts</th>
<th>Terms used</th>
<th>Hits</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>02/03/2015</td>
<td>Web of Science</td>
<td>Primary prevention of obesity by perinatal dietary interventions</td>
<td>1</td>
<td>Same as main databases</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td>Prevention of obesity by maternal dietary interventions in pregnancy</td>
<td>13</td>
<td>all results are also identified in main databases’ search</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>ETHoS</td>
<td>Prevention of obesity by maternal dietary interventions</td>
<td>1</td>
<td>Non-relevant</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>Clinicaltrials.gov</td>
<td>Prevention of obesity by maternal dietary interventions in pregnancy</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td>Primary prevention of obesity by perinatal interventions</td>
<td>10</td>
<td>Non-relevant</td>
</tr>
<tr>
<td>6</td>
<td>02/03/2015</td>
<td>ICRP**</td>
<td>Prevention of obesity (in the title) or pregnancy (in the condition) or nutrition interventions (in the intervention)**</td>
<td>631</td>
<td>Mostly non-relevant Others the same as results from main databases</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td>Maternal Health topic</td>
<td>1</td>
<td>RCT-Status: Recruiting</td>
</tr>
</tbody>
</table>

*Short phrases were used using different synonyms and for each database, the phrases that have yielded any results are shown.

**WHO International Clinical Trials Registry Platform

***Also, the list of trials by health topic, from the WHO above-mentioned platform, was looked into and trials' titles in some health topics were checked as follows:

a. Child Health (103)
b. Food Safety (3)
c. Food Insecurity (6)
d. Maternal Health (10)
e. Women’s Health (15)
## Study eligibility form - Obesity outcomes

<table>
<thead>
<tr>
<th>PICOS</th>
<th>Yes</th>
<th>Unclear</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Participants</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Are the participants classed as pregnant women, from general population, and their offspring?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Type of study</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Is the study one of the following designs during pregnancy or continued after pregnancy, either in mother or infant or both?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Randomised controlled trial</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cluster randomised controlled trial</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quasi-randomised controlled trial</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Types of interventions</strong> (the intervention could be a combination of the followings)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Food-based dietary advice (promoting a healthy diet) or nutrient intervention</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multivitamins, supplementation and minerals</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fatty acid supplement(s)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre/Probiotic supplement(s)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Outcomes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do the outcome measure obesity, on its own as a general term, or any obesity related outcome(s) i.e. anthropometry, BMI, weight gain?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Are the outcome measures validated?</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Appendix 4.3-Characteristics of the excluded studies-Obesity outcomes**

<table>
<thead>
<tr>
<th>Studies</th>
<th>Reason for exclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fatty acids</strong></td>
<td></td>
</tr>
<tr>
<td>Cheatham 2011</td>
<td>Post-natal intervention (in lactating mothers)</td>
</tr>
<tr>
<td>Courville 2011</td>
<td>Birth outcomes reported only</td>
</tr>
<tr>
<td>Haghiac 2015</td>
<td>Birth outcomes are reported and not planning for further follow-up</td>
</tr>
<tr>
<td>Innis 2008</td>
<td>Has not reported growth outcomes</td>
</tr>
<tr>
<td>Jensen 2005</td>
<td>Post-natal intervention (in lactating mothers)</td>
</tr>
<tr>
<td>Judge 2007</td>
<td>Has not reported growth outcomes</td>
</tr>
<tr>
<td>Lauritzen 2005</td>
<td>Post-natal intervention (in lactating mothers)</td>
</tr>
<tr>
<td>Makrides 2010</td>
<td>Has not reported growth outcomes</td>
</tr>
<tr>
<td>Malcolm 2003</td>
<td>Has not reported growth outcomes</td>
</tr>
<tr>
<td>Parisi 2013</td>
<td>Only abstract is available and reported early growth outcomes. Further to my personal communication, the author informed that they have not collected data on longer-term follow-up.</td>
</tr>
<tr>
<td>Sanjuro 2004</td>
<td>Maternal outcomes reported</td>
</tr>
<tr>
<td>Smithers 2011</td>
<td>Did not report growth outcomes</td>
</tr>
<tr>
<td>Smuts 2003</td>
<td>Did not report growth outcomes</td>
</tr>
<tr>
<td>Tofail 2006</td>
<td>Did not report growth outcomes</td>
</tr>
<tr>
<td><strong>Probiotics</strong></td>
<td></td>
</tr>
<tr>
<td>Allen 2014</td>
<td>Did not report growth outcomes</td>
</tr>
<tr>
<td>Boyl 2011</td>
<td>Did not report growth outcomes</td>
</tr>
<tr>
<td>Dotterud 2010</td>
<td>Did not report growth outcomes</td>
</tr>
<tr>
<td>Huurre 2008</td>
<td>Did not report growth outcomes</td>
</tr>
<tr>
<td>Kalliomäki 2001</td>
<td>Did not report growth outcomes</td>
</tr>
<tr>
<td>Kim 2010</td>
<td>Did not report growth outcomes</td>
</tr>
<tr>
<td>Kopp 2008</td>
<td>Did not report growth outcomes</td>
</tr>
<tr>
<td>Niers 2009</td>
<td>Did not report growth outcomes</td>
</tr>
<tr>
<td>Ou 2012</td>
<td>Did not report growth outcomes</td>
</tr>
<tr>
<td>Rautava 2012</td>
<td>Did not report growth outcomes</td>
</tr>
<tr>
<td><strong>Low GI diet</strong></td>
<td></td>
</tr>
<tr>
<td>Donnelly 2015</td>
<td>Infant outcomes reported within 2-3 days after birth</td>
</tr>
<tr>
<td>Koivusalo 2016</td>
<td>Maternal outcomes reported</td>
</tr>
<tr>
<td>Moses 2007</td>
<td>Non-randomised trial</td>
</tr>
<tr>
<td>Moses 2014</td>
<td>Only birth outcomes reported</td>
</tr>
<tr>
<td>Perichart-Perera 2009</td>
<td>Neonatal outcomes reported</td>
</tr>
<tr>
<td>Rhodes 2010</td>
<td>Maternal outcomes reported</td>
</tr>
<tr>
<td>Seneviratne 2014</td>
<td>Study Protocol: only exercise defined as the intervention</td>
</tr>
<tr>
<td><strong>Lifestyle change</strong></td>
<td></td>
</tr>
<tr>
<td>Adamo 2013</td>
<td>Protocol, no further updates</td>
</tr>
<tr>
<td>Althuizen 2013</td>
<td>Maternal outcomes reported</td>
</tr>
<tr>
<td>Asbee 2009</td>
<td>Maternal outcomes reported</td>
</tr>
<tr>
<td>Dodd 2016</td>
<td>Infants’ outcomes reported during their hospital stay after birth (&lt;1 month)</td>
</tr>
<tr>
<td>Ferrara 2011</td>
<td>Maternal outcomes reported</td>
</tr>
<tr>
<td>Guelinchx 2010</td>
<td>Maternal outcomes reported</td>
</tr>
<tr>
<td>Hawkins 2014</td>
<td>Maternal outcomes reported</td>
</tr>
<tr>
<td>Hui 2014</td>
<td>Birth outcomes reported (planned for a further F-U if could fund the study)</td>
</tr>
<tr>
<td>Petrella 2014</td>
<td>Maternal outcomes reported</td>
</tr>
<tr>
<td>Phelan 2011</td>
<td>Maternal outcomes reported</td>
</tr>
<tr>
<td>Polley 2002</td>
<td>Maternal outcomes reported</td>
</tr>
<tr>
<td>Poston 2015</td>
<td>Birth outcomes reported</td>
</tr>
<tr>
<td>Ruchat 2012</td>
<td>Maternal outcomes reported</td>
</tr>
<tr>
<td>Sagedal 2016</td>
<td>Birth outcomes reported</td>
</tr>
<tr>
<td>Taylor 2011</td>
<td>Postnatal intervention (in infants 0-24months)</td>
</tr>
<tr>
<td>Thomson 2016</td>
<td>Maternal outcomes reported</td>
</tr>
<tr>
<td>Thornton 2009</td>
<td>Perinatal outcomes reported</td>
</tr>
</tbody>
</table>
Vesco 2014 Maternal and birth outcomes reported
Vitolo 2011 Maternal outcomes reported
Wolff 2008 Maternal outcomes reported

Vitamins/Micronutrient
Brough 2010 Birth outcome (low income population)
Nazli 2014 Neonatal anthropometric outcomes
Parul 2013 Birth outcomes (rural Bangladesh)
Roberfroid 2008 Fetal growth (rural Burkina Faso)
Wieringa 2010 Infant’s morbidity & immune function

*Studies that were excluded at the final stage of screening are presented and also one publication, mainly a recent work for each original study is cited
**The authors may publish growth outcomes in babies in their further follow-up(s)

Fatty acid studies
- Smithers LG, Gibson RA, Makrides M. Maternal supplementation with docosahexaenoic acid during pregnancy does not affect early visual development in the infant: a randomised controlled trial. Am J Clin Nutr 2001; 93: 1293–1299

**Probiotic studies**


**Low GI diet**

- Donnelly JM, Walsh JM, Byrne J, Molloy EJ, McAuliffe FM. Impact of maternal diet on neonatal anthropometry: a randomized controlled trial. Paediatric obesity 2015; 10: 52-56.


Life-style change


Asbee SM, Jenkins TR, ButlerJR, White J, Elliot M, Rutledge A. Preventing excessive weight gain during pregnancy through dietary and lifestyle counseling: a randomized controlled trial. Obstetrics & Gynecology 2009; 113: 305–12


Vitamin/micronutrient studies

- Brough L, Rees, GA, Crawford MA, Morton Rh, Dorman EK. Effect of multiple-micronutrient supplementation on maternal nutrient status, infant birth weight


Papers published after the search strategy for these systematic reviews were updated on January 2016 (not included in the current review)

<table>
<thead>
<tr>
<th>Study</th>
<th>Intervention &amp; Outcome reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vesco 2016</td>
<td>Life-style, Obesity outcomes at 1-year age</td>
</tr>
<tr>
<td>Muhlhausler 2016</td>
<td>Fatty acid, Obesity outcomes at 3 and 5 years age</td>
</tr>
</tbody>
</table>


Ongoing studies:

- Rauh K, Kunath J, Rosenfeld E, Kick L, Ulma K, Hauner H. Healthy living in pregnancy: a cluster-randomized controlled trial to prevent excessive gestational weight gain - rationale and design of the GeliS study. BMC Pregnancy and Childbirth 2014, 14:119 [This is another on-going large-scale intervention study, named GeliS, of 2,500 pregnant women with a study population tenfold as large as the FeLIPO study (Rauh et al., 2015 which is included in the current systematic review)].
- Registered clinical trial: Life style intervention (Mediterranean diet, individual counseling and physical activity) during pregnancy for the prevention of obesity in offspring" with your name as the principle investigator. Further to my communication to Dr Calle-Pascual on September 2015, I was informed that recruitment would be completed by end of 2015. No data is published online and there was no reply to my further communication.
Appendix 4.4. Data extraction tool for obesity outcomes

- Linked Record-Do not obtain data
  Includes abstracts and later follow-up studies, provided that the later follow-up has reported the same outcome(s) for the same sample at the earlier report

- Study Details
  Country
  Recruitment Period
  Please record the months/years that the recruitment is done
  Setting
  Where the sample are taken e.g. clinics, hospital, multicentre. Please record all reported information (provide page numbers for quotes)
  Informed consent
  If not stated/unclear, please state as "NOT REPORTED/UNCLEAR"
  Ethical Approval
  If stated, please record the relevant information and tick the box.
  If not stated/unclear, please state as "NOT REPORTED/UNCLEAR"
  Source of Funding
  Please record, if stated. If not, please state "not reported".

- Trial Type
  PC-RCT
  Randomised Controlled Trial-Placebo Controlled
  Please provide brief description of study design.
  RCT
  Randomised Controlled Trial
  Please provide brief description of study design.
  CRT
  Cluster Randomised trial, please provide brief description of study design.
  Quasi Experimental
  Please provide brief description of study design.
  No. & name of Study Groups/Arms
  Please state the number and name of allocated treatment arms i.e. one treatment group vs. one placebo group, more than one treatment with/without placebo
  Please record all the relevant information briefly
  ITT conducted
  Intention-to-treat analysis
  Yes
  No
  Not mentioned
  Compliance

- Study Sample
  Matched baseline characteristics
  Yes
  No
  Women's age
  If there is any information about women's age, either stated as an inclusion criteria or other presented information, please record the age limits
  If possible, split the age data into target group, at the study start, and actual reported age in results
  Inclusion criteria
Exclusion criteria

Yes
No

Type of sample
Unselected sample
Subjects are taken from an unselected population.

Atopic history
Undernourished women
Diagnosed with GDM
Obese women
History of macrosomic baby
≥1 risk factor GDM

No. of participants at randomisation
Please record the number of PREGNANT WOMEN at the time of randomisation, where recruitment occurred prenatally.

No. at F-U

No. of subjects at the end of follow-up in both intervention and control groups.

Missing Participants
Please record the reasons missing numbers at birth e.g. number of infants eligible at birth.

Reasons Missing

Time Points Measured
Earlier follow-up(s) reported time points

Infant's age at last F-U
Please record the reported age of infants at their last follow-up

- Intervention

FA Type
Fatty acid type, please tick all relevant boxes

Any FA
LCPUFA
Long Chain Poly Unsaturated Fatty Acids
DHA
Docosahexaenoic Acid
Fish oil

Daily Dosage
Please record the total daily dosage taken in mother, infant or both, whichever is applicable with units

If more than one type is taken, please record the total daily dosage for all with units

Mode of Int. delivery during Pregnancy
Mode of Int. delivery during Infancy

Probiotic type (Organism)
Please tick all relevant boxes

Any Pro

Daily dosage
Please record the total daily dosage taken in mother, infant or both, whichever is applicable with units
If more than one type is taken, please record the total daily dosage for all with units
Mode of Int. Delivery in Infancy
Who has delivered the intervention and how e.g. mothers and oral
Mode of Int. delivery during Pregnancy e.g. oral use
Life Style Intervention
Dietary advice+ PA
any nutrition counselling plus physical activity/exercise
Dietary advice
Protein-energy supplementation
Mode of Int. intake in pregnancy
Vitamin/Suppl. Type
Vitamins/Multivitamins/Micronutrient supplements
Any Vit
Vit D3
Micronutrients
Folic Acid
Folic acid + iron
Folic acid + iron + zinc
Multiple Micronutrient
Daily Dosage
Please record the total daily taken dosage, in mother, infant or both, whichever is applicable with units
If more than on type is taken, please record the total daily dosage for all with units
Mode of Int. delivery during Pregnancy
Mode of Int. delivery in Infancy
LGI
Low Glycemic Int.
Comparisons
Placebo/Control
Standard diet
No treatment
High Fibre
Routine care
When Int. has been applied?
Pregnancy alone
Pregnancy & after delivery-In mothers
In Pregnancy & after delivery in Mothers & Infants
Pregnant women & after birth in Infants
Timing in Pregnancy
Please record intake of intervention FROM/UNTIL in pregnant women e.g. 12-40 gestation week.
Duration of Int. in Pregnancy
Please record the total duration of intake, within pregnancy e.g. 6 months
Intake IN MOTHERS after Birth
If the intervention is continued after birth in mothers, please record the intake FROM/UNTIL for that period of time e.g. 6 months after birth
Total Duration in Women

*Please record the TOTAL duration of intake in MONTHS, including after birth, if continued e.g. 7 months (36 gestation wks. + 6 months after birth)*

Timing in Infancy

*If applicable, please record the intake of intervention FROM/UNTIL in infants e.g. 6 months*

Duration in Infancy

*If applicable, please record the total duration of intake in infants after birth, in MONTHS*

Total duration of Int.

Feeding Restrictions

*If any feeding restrictions are stated in the study i.e. Breast feeding after birth, Formula feeding (quote the page number)*

Type of delivery

*Please state if not reported*

- Caesarean
- Vaginal
- Instrumental

Side Effects

- Yes
- No
- N/A-NM
  
  *Not Applicable OR Not Mentioned*

- Outcomes
  
  *The defined and measured endpoint(s) in the study*

  - Head Circumference (cm)
  - Weight (Kg)
  - Length (cm)
  - Height (cm)
  - Arm circumference (cm)
  - Waist (cm)
  - BMI (kg/m²)
  - BMI Z-score
  - Ponderal index (kg/m³)
  - Biceps [mm]
  - Triceps [mm]
  - Subscapular [mm]
  - Suprailiac [mm]
  - Sum 4 SFT [mm]

  *Sum of 4 Skinfold Thickness measurements*

  - Body fat [%]
  - Fat mass [kg]
  - LBM [g]

  *Lean Body Mass*

  - Subscapular/triceps-Ratio
  - Trunk-to-total SFT [%]

  *Trunk-to-total SFTs were calculated as (subscapular + suprailiac)/sum of 4 SFTs*100

  - Obesity
  - Overweight
Overweight/Obese
Abdominal Circumference
Hip (cm)
AC/Hip ratio
Abdominal Circumference/Hip ratio
Total fat (g)
Dual Energy X-ray (DEXA scan), in Tanvig study
Insulin (pmol/L)
Blood glucose (mmol/L)
Hb A1c fraction (%)
HOMA-IR
Homeostatic Model of Assessment of Insulin Resistance
Leptin (lg/L)
Adiponectin (microg/L)
IGF-I (microg/L)
hs-CRP (mg/L)

- Diagnosis Methods
  Please tick all that apply
  - Blood sampling
  - Anthropometric measurements
  - Please provide a brief description
  - Skinfold thickness measurements
  - Laboratory analysis
  - Please provide a brief description
  - Dietary intake
  - Body composition assessment
  - Birth measurements
  - Parkin score
  - Self-report
  - Breast milk samples
  - Follow-up interviews
  - PA measurement

- Outcome Classifications
  - Diagnosed with GDM
    Women diagnosed with Gestational Diabetes Mellitus
  - Unselected Sample
  - Undernourished women
  - Atopic history
  - Obese women
  - History of macrosomic child
### Appendix 4.5. Risk of bias judgement (fatty acid studies for prevention of obesity outcomes)

<table>
<thead>
<tr>
<th>Short Title</th>
<th>Random Sequence Generation</th>
<th>Allocation Concealment</th>
<th>Double Blinding</th>
<th>Blinding of Outcome Assessment</th>
<th>Incomplete Outcome Data</th>
<th>Selective Outcome Reporting</th>
<th>Other Sources of Bias</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bergmann (2012)</td>
<td>• Low Prospective mothers were randomized in blocks by a computer program and allocated to one of three groups (page 3, 1st column)</td>
<td>• Unclear There is no information how women are allocated</td>
<td>• Unclear There is no information whether the staff were blind at the extended F-U; however says that neither did the parents nor their children were aware of the original supplementation assignment (2012 paper, page 2, 2nd column, 4th para). Also in the original paper they report blinding but not the methods by which it was achieved.</td>
<td>• Low The examination was conducted by a pediatrician trained in anthropometric methods (K.E.B) who was not aware of the original supplementation assignment.</td>
<td>• Low 115 healthy children were examined for the 6yrs F-U which accounts for 95% of the eligible cases. This data is for children whose mothers had at least participated in one F-U visit, even if they had not regularly taken the supplement or had not exclusively breastfed for 3m.</td>
<td>• Low the secondary outcomes defined as attained growth of 6-year-old children were applied as amendment to the original study and all reported.</td>
<td>• Unclear there is no information whether the fish oil supplementation has been consumed by either the intervention or control groups, mothers or their children, after terminating the study period.</td>
</tr>
<tr>
<td>Brei (2016)</td>
<td>• Low Participants were randomly assigned on the basis of a computer-generated randomisation sequence provided by the Institute for Medical Statistics and Epidemiology, with 1:1 allocation in blocks (2012 paper, page 2)</td>
<td>• Unclear No information how allocation is done, just says randomization was performed in the 14th–15th wks. of gestation by a research assistant (2012 paper, page 2, randomisation...)</td>
<td>• High The design of the study was open-label.</td>
<td>• High Investigators who performed the measurements and analysis were not blind to the treatment.</td>
<td>• High high loss to follow-up (54.8% approached) and the most common reasons for dropout were a lack of time or relocation.</td>
<td>• Low primary &amp; secondary endpoints in methodology are reported</td>
<td>• Unclear It is not clear whether women in Int. group have consumed the FA supplement after discontinuing it at 4months.</td>
</tr>
<tr>
<td>Title</td>
<td>Generation</td>
<td>Concealment</td>
<td>Assessment</td>
<td>Outcome Data</td>
<td>Reporting</td>
<td>Bias</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
<td>-------------</td>
<td>------------</td>
<td>--------------</td>
<td>-----------</td>
<td>-----------------------------</td>
<td></td>
</tr>
<tr>
<td>Campoy (2011)</td>
<td>• Unclear</td>
<td>• High</td>
<td>• Low</td>
<td>• High</td>
<td>• Low</td>
<td>• Unclear</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Block-wise</td>
<td>Stratification by centre before the study started and participating women were randomly assigned.</td>
<td>Neither the participating women nor the study personnel knew the content of the sachets (2011 paper, page 2, study design).</td>
<td>There is not mention (i) who actually performed the outcome assessments for height etc. and (ii) whether or not they were blinded</td>
<td>between 43.6 to 53.6% of the sample have been reached for the F-U at 6.5yrs; with loss of contact and unwillingness as the main reasons for lost to F-U. There were no differences in the dropout rates between intervention groups.</td>
<td>no information as to whether the study sample have consumed FO supplements after the termination of study &amp; by the time of F-U.</td>
<td></td>
</tr>
<tr>
<td>Dunstan (2008)</td>
<td>• Unclear</td>
<td>• Low</td>
<td>• Low</td>
<td>• Low</td>
<td>• Low</td>
<td>• Unclear</td>
<td></td>
</tr>
<tr>
<td></td>
<td>just mentioned block randomisation was used according to some demographic characteristics.</td>
<td>Allocation was conducted in a blind manner and also, capsules in the two groups were image matched.</td>
<td>Mothers and research staff remained blinded until completion of the cognitive testing.</td>
<td>Of those that completed the study, 82 &amp; 90% were followed-up in the Int. &amp; placebo arms respectively. Higher lost to f-u rate was observed in the Int. arm (12 vs. 3) in the initial trial for allergy measures, because nausea in the intervention group was higher than the control (7 vs. 1).</td>
<td>the secondary outcomes reported in methodology are reported.</td>
<td>none identified</td>
<td></td>
</tr>
<tr>
<td>Short Title</td>
<td>Random Sequence Generation</td>
<td>Allocation Concealment</td>
<td>Double Blinding</td>
<td>Blinding of Outcome Assessment</td>
<td>Incomplete Outcome Data</td>
<td>Selective Outcome Reporting</td>
<td>Other Sources of Bias</td>
</tr>
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</tr>
<tr>
<td>Gonzalez-Casanova</td>
<td>Unclear</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>(2015)</td>
<td>Block randomization was used to randomly create balanced replication of four treatments (two colours for DHA and two for control) using a block size of eight.</td>
<td>The assignment codes were placed in sealed envelopes at the beginning of the study and held in a sealed location by a faculty member at Emory University (2010, page 3). Also, suppl. distributed by trained field workers during weekly visits at the participant’s homes and/or workplace (2011, page 2).</td>
<td>Researchers remained blinded to the treatment scheme until the end of the intervention, whereas participants and field personnel remain blinded to date.</td>
<td>Researchers remained blinded to the treatment scheme until the end of the intervention, whereas participants and field personnel remain blinded to date.</td>
<td>Low 73.67 and 72.94% of participants in the intervention and placebo groups were followed-up respectively, which is below 80% of the satisfactory participation rate.</td>
<td>Low The primary outcome measures defined in methodology are reported.</td>
<td>Low There were no significant differences in reported consumption of any DHA-source foods by offspring between treatment groups at either time point.</td>
</tr>
<tr>
<td>Helland (2008)</td>
<td>Low</td>
<td>Unclear</td>
<td>Unclear</td>
<td>Unclear</td>
<td>High</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td></td>
<td>The randomization was performed by a computer program (2001 paper, materials &amp; methods, 2nd column)</td>
<td>No information about the method of allocation (in 2001 or 2008 papers)</td>
<td>For the outcomes of interest, the measurements were taken in routine clinic appointments where it is unlikely the staff knew about the study and parents then sent a copy of the appropriate information to study staff. So, since it is unclear if parents were appropriately blinded, it is unclear whether there could have had an influence on the outcome assessment.</td>
<td>For the outcomes of interest, the measurements were taken in routine clinic appointments where it is unlikely the staff knew about the study and parents then sent a copy of the appropriate information to study staff. So, since it is unclear if parents were appropriately blinded, it is unclear whether there could have had an influence on the outcome assessment.</td>
<td>Loss to F-U is high (&lt;50% of the invited infants followed up during the 1st year of their life have attended the F-U at 7yrs age). Also the non-compliance rate was higher in the Int. vs. control group.</td>
<td>Low the primary and secondary endpoints defined in methodology are reported.</td>
<td>Low There was no statistical difference between the groups concerning intake of cod liver oil during the preschool age. (based on mothers’ report).</td>
</tr>
<tr>
<td>Short Title</td>
<td>Random Sequence Generation</td>
<td>Allocation Concealment</td>
<td>Double Blinding</td>
<td>Blinding of Outcome Assessment</td>
<td>Incomplete Outcome Data</td>
<td>Selective Outcome Reporting</td>
<td>Other Sources of Bias</td>
</tr>
<tr>
<td>-------------</td>
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</tr>
<tr>
<td>Rytter (2011)</td>
<td>• Unclear Women were randomly assigned to the three groups in the ratio 2/1/1. Randomisation was stratified by parity (no previous full-term childbirth vs one or more) and arranged in balanced blocks of between 8 and 12 (Olsen 1992, page 4, 1st para)</td>
<td>• Low Randomisation numbers for each study number were kept in a sealed, opaque envelope that either identified a particular package of oil capsules or no oil supplement. The capsules and their boxes looked identical (Olsen 1992, page 4, 1st para).</td>
<td>• High Women would know if they were in the control group as they would not receive any tablets. This would very much depend on what information was provided to participants. If they are fully informed (i.e. in order to give proper consent) then they should have been told what being part of the control group would involve. Does not report blinding of investigators.</td>
<td>• Low The 2 assistants, who made all the measurements, were blinded to group allocation (2011 paper, page 2, subjects, 2nd para)</td>
<td>• High High loss to F-U which is bigger in fish oil group, mostly due to refusing, not completing the clinical examinations and no responses.</td>
<td>• Low All the defined outcomes mentioned in the methods are reported.</td>
<td>• Low None identified (The 3 groups were similar with respect to most covariates. The only difference that was statistically significant was the smoking status among the offspring, with smoking being more prevalent in participants from the olive oil group than in the other 2 groups (Table 2)).</td>
</tr>
</tbody>
</table>
### Appendix 4.6. Risk of bias judgement (probiotic studies for prevention of obesity outcomes)

<table>
<thead>
<tr>
<th>Short title</th>
<th>Random Sequence Generation</th>
<th>Allocation Concealment</th>
<th>Double Blinding</th>
<th>Blinding of Outcome Assessment</th>
<th>Incomplete Outcome Data</th>
<th>Selective Outcome Reporting</th>
<th>Other Sources of Bias</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abrahamsson (2013)</td>
<td>• Unclear Randomisation was stratified for each study centre. No more information is given</td>
<td>• Low Each centre was provided an allocation list with unique ID numbers for each subject. Prior to randomisation, each study product bottle was labelled with the unique ID number and randomly mixed by an independent contract manufacturer.</td>
<td>• High The study was conducted in a double blind fashion until all infants had completed the 2yrs follow-up.</td>
<td>• High The study was conducted in a double blind fashion until all infants had completed the 2y F-U.</td>
<td>• High There was high loss to follow-up, but equally spread across both groups. However, reasons for being lost to follow-up are not reported.</td>
<td>• Unclear Primarily designed to assess allergic outcomes in children and obesity measures reported as secondary outcomes</td>
<td>• High Infants in the placebo group did not receive the supplement after birth. Also, At 7y of age, 19% vs. 26% (L. reuteri vs. placebo) reported had taken any probiotic strain in the last month (p=0.30).</td>
</tr>
<tr>
<td>Kuitunen (2009)</td>
<td>• Low Computer-generated block randomisation</td>
<td>• Low Throughout the study, the randomisation code was kept by the database consultant and revealed to the statistician only. The capsules and syrups, looked, smelled, and tasted identical</td>
<td>• Unclear No statement as to whether the paediatrician was blinded to the treatment allocation at 5yrs F-U</td>
<td>• Unclear A high number of mothers in both groups refused to participate and also, reasons for lost to follow-up not specified.</td>
<td>• Unclear Primarily designed to assess allergic outcomes in children and obesity measures reported as secondary outcomes</td>
<td>• Low non identified</td>
<td></td>
</tr>
<tr>
<td>Luoto (2010)</td>
<td>• Unclear Says women in the intervention group were randomized in a double-blind manner to receive either probiotic or placebo capsules</td>
<td>• Unclear no information is provided</td>
<td>• Low During the intervention period neither parents nor clinicians were aware of allocation, and the intervention/comparison capsules were similar in appearance, taste and</td>
<td>• Low The clinician was un-blinded at termination of the original study (2001 paper). However, the follow-up data was</td>
<td>• Unclear Reasons for lost to F-U are not provided</td>
<td>• Low The defined outcomes in methodology are reported</td>
<td>• Unclear no information whether children in the Int. group have consumed probiotics after the termination</td>
</tr>
</tbody>
</table>
with no more details

smell (2001 paper).

collected from parents, having been measured by school nurses, and it seems likely blinding was still in place for parents and that school nurses were also unaware of allocation.

| Wickens (2013) | • Low Randomisation was stratified by study centre and performed in blocks of 15 according to a computer-generated randomisation list. | • Low Randomisation and allocation of supplements were performed by a clinical trials pharmacist with no contact with the participants. | • High After the 2-year follow-up parents were not blind to study group, and were subsequently asked to report subjectively on eczematous symptoms. | • Low Study nurses remained blinded to participant study group through-out | • High Imputed analysis is used for some of the reported outcomes; also the reasons for lost to F-U are not specified. | • Unclear Primarily designed to assess allergic outcomes in children and obesity measures reported as secondary outcomes | • Low Infants excluded if exposed to commercially available non-study probiotics, either directly or through breast milk during the course of the study |
Appendix 4.7. Risk of bias judgement (LG index diet studies for prevention of obesity outcomes)

<table>
<thead>
<tr>
<th>Short Title</th>
<th>Random Sequence Generation</th>
<th>Allocation Concealment</th>
<th>Double Blinding</th>
<th>Blinding of Outcome Assessment</th>
<th>Incomplete Outcome Data</th>
<th>Selective Outcome Reporting</th>
<th>Other Sources of Bias</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horan 2016</td>
<td>• Low Using computer generated allocations in a ratio of one to one (Walsh, 2012, patient selection)</td>
<td>• Unclear The research midwife did the randomisation in sealed opaque envelopes (Walsh 2012, patient selection)</td>
<td>• High participants were aware of the type of intervention</td>
<td>• Unclear There is no indication whether the measurements were conducted in a blind fashion (Horan 2016, 2.3 section).</td>
<td>• High Only 280 women (35%) returned for the 6months postpartum F-U and completed food diaries &amp; other questionnaires. (2016 paper, page3, 2.2 part)</td>
<td>• Low [Info] All predefined outcomes in methodology are reported.</td>
<td>• High No detailed dietary data was collected at 6ms postpartum, so no information whether women continued the low GI diet or reverted to pre-pregnancy dietary habits.</td>
</tr>
<tr>
<td>Louie 2013</td>
<td>• Low women were centrally randomised to study diet by computer-generated random numbers, stratified by BMI (BMI &lt;30 vs. &gt;=30 kg/m2) and weeks of gestation (&lt;28 or &gt;=28 weeks).</td>
<td>• Unclear Says the allocation sequence was unpredictable and concealed from the recruiter, but does not say the method by which they achieved this (2011 paper, page 2, Subject recruitment...)</td>
<td>• Unclear No information regarding the blinding of research staff and participants, also the study dieticians may well have known the allocation given that they were being asked to judge compliance with the diets based on 24hr food diaries. Whether they communicated this to participants or other study staff is unclear.</td>
<td>• Unclear A biostatistician blinded to the diet allocation performed the statistical analyses at birth, but no information is provided for the F-U at 3mon.</td>
<td>• High 59% of the original cohort returned for F-U with no reasons mentioned for the attrition.</td>
<td>• Low All the pre-defined outcomes in methodology are reported.</td>
<td>• Unclear The post-partum diet was not formally assessed. Mothers were encouraged to continue their assigned diet; however, it is possible that they reverted to their habitual eating pattern</td>
</tr>
</tbody>
</table>
### Appendix 4.8. Risk of bias judgement (life-style change studies for prevention of obesity outcomes)

<table>
<thead>
<tr>
<th>Short Title</th>
<th>Random Sequence Generation</th>
<th>Allocation Concealment</th>
<th>Double Blinding</th>
<th>Blinding of Outcome Assessment</th>
<th>Incomplete Outcome Data</th>
<th>Selective Outcome Reporting</th>
<th>Other Sources of Bias</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rauh 2015</td>
<td>• Low Randomisation was performed at the cluster level, i.e. gynecological practices were randomised (rather than individuals) using a computer-generated randomisation allocation table (2013 paper).</td>
<td>• Low Randomisation was performed by a researcher not involved in the study design thereby preventing allocation bias (2013 paper).</td>
<td>• High The nature of the study meant that participants and study staff were not blinded to the types of intervention (2013 paper, page 2).</td>
<td>• High More women participated in practices allocated to the intervention group than in control practices (12 vs. 22%), causing unequal group sizes.</td>
<td>• High</td>
<td>• Low All the pre-defined outcomes in methodology are reported.</td>
<td>• Unclear The dietary intake &amp; PA level are not assessed after delivery and its not clear whether mother adhered the consulted intervention.</td>
</tr>
<tr>
<td>Tanvig (2015)</td>
<td>• Low Women were randomised using computer-generated No. in closed envelopes (page 11, 2014 report)</td>
<td>• Unclear A doctor and a research midwife enrolled the patients and women themselves picked-up their randomisation number from a basket and opened (page 11, 2014 report).</td>
<td>• High There was no blinding to patients, care givers or the doctor (page 11, 2014 report)</td>
<td>• Low All children were examined by the same medical doctor (M.T.), blinded to the RCT Int. Information on who had received intervention was revealed after data collection was complete.</td>
<td>• High Less than 50% are approached with no reasons mentioned for lost to F-U at 2.8yrs (Figure 1)</td>
<td>• Low The pre-defined outcomes in methodology are reported</td>
<td>• Unclear Unclear if mother in the either study group have conducted any other exercise e.g. exercise at home, short length walking/brisk</td>
</tr>
</tbody>
</table>
Dear mother/parent

You have received this questionnaire as part of the Portsmouth Birth Cohort Registry. Here, we would like to ask you about the foods you eat. To do this, there is a list of foods and we would like you to tell us how often you have eaten each food during the past 3 months, by ticking the boxes. The list may include foods you never eat or you may find foods which you eat a lot are missing. These can be added at the end. This questionnaire should take 10-15 minutes to complete.

We will use the information from this questionnaire to see if there is any relationship between what women eat when they are pregnant and the development of allergies in their babies or how quickly their babies grow. We very much appreciate your time completing the questionnaire. Please return the completed questionnaire using the enclosed pre-paid addressed envelope. The return of the questionnaire will be considered as your consent.

If you have any queries, please do contact me in relation to my role as Principal Investigator for this study.

Yours faithfully

Professor Tara Dean
Dean of Science
Portsmouth Birth Cohort Study

1. Are you currently excluding any foods from your diet?  
   Yes  No
   IF ‘NO’ GO TO Q. 4

2. If yes, why? Please specify, even if because of morning sickness

3. What food group(s) are you excluding? Please specify

4. Have you taken any medication (e.g. antibiotics, paracetamol or aspirin) during your pregnancy so far?  
   Yes  No

5. If yes, what? (If no tick assume answer to be NO)

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibiotics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paracetamol</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Aspirin</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Other medication</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Do you take any nutritional supplements e.g. Vitamins?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

6. Do you know how much you weighed before pregnancy?  
   Yes  No

7. If yes, please state (kg)

8. How much attention do you pay to your diet in terms of healthy eating?  
   Very little  Somewhat  A great deal
**Food Frequency Questionnaire** *(Define the 3 month period)*

*On average how often have you eaten the following foods during the last three months? (please tick)*

<table>
<thead>
<tr>
<th>FOOD CODE</th>
<th>FOOD DESCRIPTION</th>
<th>FREQUENCY EATEN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>STARCHY CARBOHYDRATES</td>
<td>Never</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Once every 2-3 Months</td>
</tr>
<tr>
<td>1</td>
<td>White bread</td>
<td></td>
</tr>
<tr>
<td></td>
<td>When you eat bread/toast/sandwiches, how many slices/rolls do you eat at a typical meal?</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rolls <em>count as 2 slices</em> French bread <em>baguette</em> <em>2″ counts as 1 slice</em></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Brown and wholemeal bread/rolls</td>
<td></td>
</tr>
<tr>
<td></td>
<td>How many slices/rolls do you eat at a typical meal?</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rolls <em>count as 2 slices</em></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Crackers</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Wholemeal and rye crackers</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>High fibre breakfast cereals (e.g. Weetabix)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Other breakfast cereals</td>
<td></td>
</tr>
<tr>
<td>FOOD CODE</td>
<td>FOOD DESCRIPTION</td>
<td>FREQUENCY EATEN</td>
</tr>
<tr>
<td>-----------</td>
<td>------------------</td>
<td>-----------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Never</td>
</tr>
<tr>
<td>7</td>
<td>Added bran to foods</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Potatoes boiled and jacket</td>
<td></td>
</tr>
<tr>
<td></td>
<td>When you eat these how many potatoes do you eat at a typical meal?</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Large baking (count as 3)/new (count as 0.5)</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Roast potatoes and chips</td>
<td></td>
</tr>
<tr>
<td></td>
<td>When you eat these how many potatoes do you eat at a typical meal?</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Yorkshire puddings and savoury pancakes</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>White rice</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Brown rice</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Pasta</td>
<td></td>
</tr>
<tr>
<td>FOOD CODE</td>
<td>FOOD DESCRIPTION</td>
<td>FREQUENCY EATEN</td>
</tr>
<tr>
<td>-----------</td>
<td>----------------------------------------</td>
<td>-----------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Never</td>
</tr>
<tr>
<td>14</td>
<td>Peas and green beans</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Carrots</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Parsnips, swede and turnip</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Sweetcorn and mixed veg</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>Beans and pulses</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>Tomatoes</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>Spinach</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>Broccoli, Brussels sprouts and spring greens</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>Cabbage and cauliflower</td>
<td></td>
</tr>
<tr>
<td>FOOD DESCRIPTION</td>
<td>FREQUENCY EATEN</td>
<td></td>
</tr>
<tr>
<td>---------------------------</td>
<td>-----------------</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Never</td>
<td>Once every 2-3 Months</td>
</tr>
<tr>
<td>23 Peppers and watercress</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 Onion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 Green salad</td>
<td></td>
<td></td>
</tr>
<tr>
<td>26 Side salads in dressing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>27 Courgettes, marrow and leeks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28 Mushrooms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>29 Vegetable dishes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 Soup</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FOOD CODE</td>
<td>FOOD DESCRIPTION</td>
<td>FREQUENCY EATEN</td>
</tr>
<tr>
<td>-----------</td>
<td>------------------</td>
<td>----------------</td>
</tr>
<tr>
<td>31</td>
<td>Fresh apples and pears</td>
<td>Never, Once every 2-3 Months, Once a Month, 1-2 Times per Week</td>
</tr>
<tr>
<td>32</td>
<td>Bananas</td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>Strawberries, raspberries and blueberries</td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>Fresh peaches, plums, cherries and grapes</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>Fresh pineapple, melon, kiwi fruit and other tropical fruits</td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>Fresh oranges and orange juice</td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>Tinned fruit not including grapefruit, prunes, figs or blackcurrants</td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>Cooked fruit not including blackcurrants</td>
<td></td>
</tr>
<tr>
<td>39</td>
<td>Dried fruit</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>Grapefruit and grapefruit juice</td>
<td></td>
</tr>
<tr>
<td>41</td>
<td>Blackcurrants, Ribena and hi-juice blackcurrant drinks</td>
<td></td>
</tr>
<tr>
<td>FOOD CODE</td>
<td>FOOD DESCRIPTION</td>
<td>FREQUENCY EATEN</td>
</tr>
<tr>
<td>-----------</td>
<td>---------------------------------------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td>42</td>
<td>Cola drinks</td>
<td>Never</td>
</tr>
<tr>
<td>43</td>
<td>Diet cola drinks</td>
<td>Once every 2-3 Months</td>
</tr>
<tr>
<td>44</td>
<td>Soft drinks not including diet drinks</td>
<td>Once a Month</td>
</tr>
<tr>
<td>45</td>
<td>Energy drinks</td>
<td>Once a Fortnight</td>
</tr>
<tr>
<td>46</td>
<td>Water</td>
<td>1-2 Times per Week</td>
</tr>
<tr>
<td>47</td>
<td>Alcohol</td>
<td>3-6 Times per Week</td>
</tr>
<tr>
<td>48</td>
<td>Tea</td>
<td>Once a day</td>
</tr>
<tr>
<td>49</td>
<td>Coffee</td>
<td>More than once a day</td>
</tr>
<tr>
<td>50</td>
<td>Decaffeinated tea or coffee</td>
<td></td>
</tr>
<tr>
<td>51</td>
<td>Drinking chocolate and milk shakes not including McDonalds style milkshakes</td>
<td></td>
</tr>
<tr>
<td>FOOD CODE</td>
<td>MEAT AND SUBSTITUTES</td>
<td>FOOD DESCRIPTION</td>
</tr>
<tr>
<td>-----------</td>
<td>------------------------------------------</td>
<td>------------------------------------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Never</td>
</tr>
<tr>
<td>52</td>
<td>Bacon and gammon</td>
<td></td>
</tr>
<tr>
<td>53</td>
<td>Pork (e.g. pork chop)</td>
<td></td>
</tr>
<tr>
<td>54</td>
<td>Sausages</td>
<td></td>
</tr>
<tr>
<td>55</td>
<td>Ham or luncheon meat</td>
<td></td>
</tr>
<tr>
<td>56</td>
<td>Chicken and turkey breast</td>
<td></td>
</tr>
<tr>
<td>57</td>
<td>Chicken and turkey in breadcrumbs</td>
<td></td>
</tr>
<tr>
<td>58</td>
<td>Lamb</td>
<td></td>
</tr>
<tr>
<td>59</td>
<td>Beef</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>Minced meat dishes (e.g. lasagne, bolognaise)</td>
<td></td>
</tr>
<tr>
<td>61</td>
<td>Meat pies</td>
<td></td>
</tr>
<tr>
<td>FOOD CODE</td>
<td>FOOD DESCRIPTION</td>
<td>FREQUENCY EATEN</td>
</tr>
<tr>
<td>-----------</td>
<td>------------------</td>
<td>----------------</td>
</tr>
<tr>
<td></td>
<td>FOOD DESCRIPTION</td>
<td>Never</td>
</tr>
<tr>
<td>62</td>
<td>Liver and kidney</td>
<td></td>
</tr>
<tr>
<td>63</td>
<td>Paté and liver sausage</td>
<td></td>
</tr>
<tr>
<td>64</td>
<td>Vegetarian substitutes (e.g. Quorn)</td>
<td></td>
</tr>
<tr>
<td>65</td>
<td>Take away/fast food (e.g. KFC, McDonalds)</td>
<td></td>
</tr>
<tr>
<td>66</td>
<td>Ready meals (e.g. pot noodles, microwave meals)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>FOOD CODE</th>
<th>FOOD DESCRIPTION</th>
<th>FREQUENCY EATEN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FOOD DESCRIPTION</td>
<td>Never</td>
</tr>
<tr>
<td>67</td>
<td>Fish in breadcrumbs</td>
<td></td>
</tr>
<tr>
<td>68</td>
<td>White fish not in breadcrumbs</td>
<td></td>
</tr>
<tr>
<td>69</td>
<td>Oily fish (e.g. Salmon, Mackerel, Sardines)</td>
<td></td>
</tr>
</tbody>
</table>
70 | Shellfish |
---|---|
71 | Boiled and poached eggs |
72 | Omelette and fried eggs |
73 | Cottage Cheese |
74 | Cheese |
75 | Pizza, quiches and cheese flans |

<table>
<thead>
<tr>
<th>FOOD CODE</th>
<th>FOOD DESCRIPTION</th>
<th>FREQUENCY EATEN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Never</td>
</tr>
<tr>
<td>76</td>
<td>Yoghurt and fruit fools</td>
<td></td>
</tr>
<tr>
<td>77</td>
<td>Cream</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Less than ½ a cup (&lt;75 ml)</td>
</tr>
<tr>
<td>78</td>
<td>On average how much milk do you consume per day (including milk in coffee, tea, cereal)?</td>
<td></td>
</tr>
</tbody>
</table>
Which type of milk do you predominantly drink? Circle one

<table>
<thead>
<tr>
<th>FOOD CODE</th>
<th>FOOD DESCRIPTION</th>
<th>SWEET AND MISCELLANEOUS FOODS</th>
<th>FREQUENCY EATEN</th>
</tr>
</thead>
<tbody>
<tr>
<td>79</td>
<td></td>
<td>Skimmed or 1%</td>
<td>Semi skimmed</td>
</tr>
<tr>
<td>80</td>
<td>Milk based puddings (e.g. custard)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>81</td>
<td>Ice cream</td>
<td></td>
<td></td>
</tr>
<tr>
<td>82</td>
<td>Chocolate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>83</td>
<td>Other sweets (not chocolate)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>84</td>
<td>Cakes and gateaux</td>
<td></td>
<td></td>
</tr>
<tr>
<td>85</td>
<td>Buns</td>
<td></td>
<td></td>
</tr>
<tr>
<td>86</td>
<td>Pastries</td>
<td></td>
<td></td>
</tr>
<tr>
<td>87</td>
<td>Chocolate or digestive biscuits</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FOOD CODE</td>
<td>FOOD DESCRIPTION</td>
<td>FREQUENCY EATEN</td>
<td></td>
</tr>
<tr>
<td>-----------</td>
<td>----------------------------------------------------------</td>
<td>-----------------</td>
<td></td>
</tr>
<tr>
<td>88</td>
<td>Other biscuits</td>
<td></td>
<td></td>
</tr>
<tr>
<td>89</td>
<td>Sweet spreads (e.g. jam/honey/chocolate spread)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>Added sugar (e.g. added to cereal, coffee, tea)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>91</td>
<td>Gravy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>92</td>
<td>Stock cubes or marmite</td>
<td></td>
<td></td>
</tr>
<tr>
<td>93</td>
<td>Mayonnaise and salad cream</td>
<td></td>
<td></td>
</tr>
<tr>
<td>94</td>
<td>Pickles, chutney, tomato ketchup and brown sauce</td>
<td></td>
<td></td>
</tr>
<tr>
<td>95</td>
<td>Crisps and savoury snacks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>96</td>
<td>Nuts</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FOOD CODE</td>
<td>FOOD DESCRIPTION</td>
<td>FREQUENCY EATEN</td>
<td></td>
</tr>
<tr>
<td>-----------</td>
<td>--------------------------------------------------------</td>
<td>-----------------</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Never</td>
<td>Once every 2-3 Months</td>
</tr>
<tr>
<td>97</td>
<td>Spreading fat (please specify)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>98</td>
<td>Frying fat or oil (please specify)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>99</td>
<td>Other vegetable oil e.g. salad dressings, marinades (please specify)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Are there food or drinks which you have eaten or drunk **once a week or more** which are not on the list?  

*If Yes*  

<table>
<thead>
<tr>
<th>No</th>
<th>Yes</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Name of food/drink</th>
<th>1-2 times per week</th>
<th>3-6 times per week</th>
<th>Once a day</th>
<th>More than once a day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

444
Dear parent

You have received this questionnaire as part of the Portsmouth Birth Cohort Registry. In this questionnaire will ask you about what your baby is eating and drinking. We want to find out whether the foods and drinks that babies have when they are very young affects how quickly they grow or whether they develop allergies later in life. We will ask you about milk(s) that your baby has during the next 4 days. You can start any day of the week and carry on for four days. All you need to do is as follows:

- Simply write down ANY MILK your infant has during the next 4 days e.g. a breastfeed, formula milk or both breast/formula.
- If breastfed, please write down the approximate duration of the feed (e.g. 15 minutes).
- If a formula feed, please give details of what is given (e.g. brand and type of milk). Please also provide details of the amount taken e.g. 4floz or 120mls
- If your baby has any other liquids in the next four days (e.g. water), please also write them down.

We very much appreciate your time completing the questionnaire. Please return the completed questionnaire, using the enclosed pre-paid addressed envelope. The return of the questionnaire will be considered as your consent. If you have any queries, please do contact me in relation to my role as Principal Investigator for this study.

Yours faithfully

Tara Dean
Professor Tara Dean
Dean of Science
Please answer these questions before completing the diary:

1. What is your baby’s current or most recent weight (please look in your red book)?
   - Weight: ____________________
   - Date weighed: _______________

2. Have you introduced any formula feeds to your baby’s diet? (if no please go to Q3)
   - Yes □  No □
   If yes,
      2.1 How old was your baby when you first introduced formula?
      _______________

2.2 What type of formula do you feed your baby?
   - Regular infant milk such as SMA, Aptamil etc.
     (please specify)
   - Specialised formula (please specify)

3. Have you introduced any other drinks (including water)? (if no please go to Q4)
   - Yes □  No □
   If yes, have you given any of the following drinks to your baby? And how old was your baby when you first gave them these drinks?

<table>
<thead>
<tr>
<th>Drink</th>
<th>Never Given</th>
<th>&lt; 1 month</th>
<th>1-2 months</th>
<th>2-3 months</th>
<th>&gt;3 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baby Juice</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fruit Juice</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Squash (not low calorie or low sugar)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low-calorie or low sugar</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Do you ever add anything into your baby’s bottle e.g. rusk or baby rice?  
If yes, please specify (if no please go to Q5)

Have you introduced any solids into your baby’s diet?  
If yes, please specify (if no please go to Q6)

How much did you weigh at the end of your pregnancy (if known)?
Please record what your baby actually drank, not what was offered to them. Please remember to include liquids given by any other carer/family member

<table>
<thead>
<tr>
<th>Milk Diary Started on:</th>
<th>Morning 5am-12pm</th>
<th>Afternoon 12pm-4pm</th>
<th>Evening 4pm-10pm</th>
<th>Night 10pm-5am</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Example:

<table>
<thead>
<tr>
<th>Milk Diary Started on:</th>
<th>Tuesday 10(^{th}) May</th>
<th>(Please add date)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>4oz Aptamil First formula</td>
<td>15 minute breast feed</td>
</tr>
<tr>
<td></td>
<td>10 minute breastfeed x 2</td>
<td>20 minute breastfeed x 1</td>
</tr>
<tr>
<td></td>
<td>30ml water</td>
<td>4oz Aptamil First formula</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3oz Aptamil First formula</td>
</tr>
</tbody>
</table>
PARTICIPANT INFORMATION SHEET
PORTSMOUTH BIRTH COHORT REGISTRY

Researchers
Professor Tara Dean, Epidemiology and Health Services Research
Dr Saseendran Pallikadavath, Health Demographer
Senior Research Fellows: Dr Suzannah Helps, Dr Kate Maslin
Research Midwives: Zoe Garner, Jess Madgwick, Linda Lishman
Database Manager: Jill Glasby

What is a birth cohort registry?
It is a register (list) of children born during a specified time period. Details of health and other information are collected for each child born in the particular time period and these details are added into an electronic database. In this case the database will include all Portsmouth children born during 2015-2016 to women who have consented to be included. Registered children would be on the database from birth onwards, for the rest of their lives. However, they can, from the age of 16, or earlier if their mother wishes, withdraw from the register at any time without giving any reason.

Why do we need a birth cohort registry in Portsmouth?
Portsmouth is different from other cities in the South of England. The overall health of people living in Portsmouth is worse than the average for England. To help health, social, and education services provide what is needed for people of the city, they need good information; at the moment there is not enough information to base policies on and plan services for the local population in Portsmouth. The information in the register would be used by researchers to help find out what services the local people need.

Why have I been chosen?
You have been chosen because you are pregnant and your baby will be born in Portsmouth in the next 12 months. We want to study things like how your diet, social environment, and health status influence the health of your child so we would like to ask you some questions about yourself and ask you whether you would consent for your child and his or her information to be included in the register. Also we need your permission to register your child with the birth cohort so that we can study how children in Portsmouth grow and develop over the years.

As part of this study you will be approached for an interview to discuss issues such as your health, diet and social circumstances. If you are happy to take part in this interview you will be asked to sign the consent form.
We will also ask your permission to register your child with the birth cohort registry and obtain health information on your child from the hospital at birth.

In order to understand more about your child we will need to ask about his or her father and his or her brothers and sisters. We will be asking you to make sure that the baby’s father is happy for you to provide information about him. As you will be the responsible person to sign the consent for the child till she/he reaches sufficient maturity to decide for themselves at age 16 and after, we will be asking your consent to approach you for any future study.

**Do I have to take part in the cohort registry?**

No. You do not have to take part in the registry. It is voluntary. Even if you take part you are completely free to leave the registry at any time. You will not be asked for your reasons. If you do decide to leave the registry we will still use the information that we have already collected (with your name and any information that might let people identify you removed), unless you ask us to delete all of your data from the registry.

**What does the registry mean to me?**

The registry will help the researchers at the University of Portsmouth to contribute to policies and programmes of ‘Portsmouth City’ which includes the Portsmouth City Council and Portsmouth NHS organisations. Birth cohort information will be an important resource for answering many questions which eventually contribute to scientific knowledge.

**What are the benefits of taking part?**

There is no direct benefit to you. However, if we find any health or social issues related to you and your family we will advise you to get appropriate help/support.

**What are the possible risks or disadvantages of taking part?**

There is minimal risk in participating in this registry. Your child will be in the registry as long as you wish. If you have given consent you will be approached, until the child reaches maturity at age 16 years, to see whether you would be interested in associated future studies and further information collection which may occur every 3-4 years. On maturity, at age 16, your child will be responsible for giving consent to any future study and information collection. Each time your child is invited to participate in future studies, we will make sure we have appropriate ethical approval.

**Will my participation be confidential?**

Yes. The part of the registry which can be accessed by researchers will have no information which could be used to identify you. All identifiable information will be stored in a Master file with access only to the Chief Investigator and Data Manager. We may share anonymised data with academic researchers with approval from the data management committee and ethical approval from local research ethics committees.

**What if I do have any questions?**

You can contact us using the study email (PortsmouthBirthCohort@port.ac.uk) or our
phone number 07738688089. You can also ask questions through the dedicated website for this study - the Portsmouth birth cohort registry.

www.port.ac.uk/portsmouthbirthcohort

What happens now?

If you are willing to participate in the registry please complete the consent form. You can either give it to the Midwife now or return it at your next antenatal visit.

Thank you for considering this study.
Appendix 5.4. Study consent form

PORTSMOUTH BIRTH COHORT REGISTRY

Reference number: 15/SC/0008

Participant Identification Number for this study: ………………………………………………

CONSENT FORM

Title of Project: PORTSMOUTH BIRTH COHORT REGISTRY

Name of Researcher: Professor Tara Dean

Please initial each box

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I confirm that I have read and understood the information sheet version 4 dated 28/10/2015 for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.</td>
</tr>
<tr>
<td>2</td>
<td>I understand that my participation is voluntary and that I am free to withdraw at any time.</td>
</tr>
<tr>
<td>3</td>
<td>I understand that relevant sections of my medical notes and data collected during the information collection, may be looked at by individuals from the University of Portsmouth, from regulatory authorities or from the NHS Trust, where it is relevant. I give permission for these individuals to have access to my records.</td>
</tr>
<tr>
<td>4</td>
<td>I understand that data collected during the study may be used for research by responsible individuals from the University of Portsmouth and other university approved researchers.</td>
</tr>
<tr>
<td>5</td>
<td>I understand that I may be contacted (until my child reaches maturity, at age 16), should the researchers plan to undertake any studies or collect more information and my consent will be obtained before undertaking any interviews regarding my child.</td>
</tr>
<tr>
<td>6</td>
<td>I have discussed the research with the child’s father and he understands that I will be asked to answer questions relating to him, the father and the baby’s siblings. I understand that, sibling details will be asked only if I am also their biological parent.</td>
</tr>
<tr>
<td>7</td>
<td>I agree to my child going into the registry.</td>
</tr>
</tbody>
</table>

________________________  __________________  __________________
Name of Participant  Date  Signature

________________________  __________________
Name of Person taking consent  Date  Signature
(if different from researcher)

________________________  __________________
Researcher  Date  Signature

When completed, 1 for participant; 1 for researcher site file
Appendix 5.5. Ethical approval letter for the Portsmouth Birth Cohort (PBC) registry from Berkshire NHS

23 January 2015

Professor Taran is (Tara) Dean
University of Portsmouth
Purple Door, 28 Guildhall Walk
Portsmouth
PO1 2DD

Dear Professor Dean

Title of the Research Database: Portsmouth Birth Cohort Registry
REC reference: 15/SC/0008
IRAS project ID: 162850

Thank you for your letter responding to the Committee’s request for further information on the above research database and submitting revised documentation.

The further information has been considered on behalf of the Committee by the the Chair.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this favourable opinion letter. The expectation is that this information will be published for all studies that receive an ethical opinion but should you wish to provide a substitute contact point, wish to make a request to defer, or require further information, please contact the REC manager, Miss Lauren Allen, nrescommittee.southcentral-berkshireB@nhs.net. Under very limited circumstances (e.g. for student research which has received an unfavourable opinion), it may be possible to grant an exemption to the publication of the study.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion of the above research database on the basis described in the application form and supporting documentation as revised.

Duration of ethical opinion

The favourable opinion is given for a period of five years from the date of this letter and provided that you comply with the standard conditions of ethical approval for Research Databases set out in the attached document. You are advised to study the conditions carefully. The opinion may be renewed for a further period of up to five years on receipt of a fresh application. It is
suggested that the fresh application is made 3-6 months before the 5 years expires, to ensure continuous approval for the research database.

Approved documents

The documents reviewed and approved at the meeting were:

<table>
<thead>
<tr>
<th>Document</th>
<th>Version</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Covering letter on headed paper [Covering letter]</td>
<td>1</td>
<td>12 December 2014</td>
</tr>
<tr>
<td>Other [Protocol]</td>
<td>1</td>
<td>12 December 2014</td>
</tr>
<tr>
<td>Other [CV]</td>
<td>1</td>
<td>12 December 2014</td>
</tr>
<tr>
<td>Other [Wave 0 questionnaire clean]</td>
<td>2</td>
<td>22 January 2015</td>
</tr>
<tr>
<td>Other [Wave 1 questionnaire clean]</td>
<td>2</td>
<td>22 January 2015</td>
</tr>
<tr>
<td>Participant consent form</td>
<td>3</td>
<td>23 January 2015</td>
</tr>
<tr>
<td>Participant information sheet (PIS)</td>
<td>3</td>
<td>23 January 2015</td>
</tr>
<tr>
<td>REC Application Form [RD_Form_15122014]</td>
<td></td>
<td>15 December 2014</td>
</tr>
</tbody>
</table>

Research governance

Under the Research Governance Framework (RGF), there is no requirement for NHS research permission for the establishment of research databases in the NHS. Applications to NHS R&D offices through IRAS are not required as all NHS organisations are expected to have included management review in the process of establishing the database.

Research permission is also not required by collaborators at data collection centres (DCCs) who provide data under the terms of a supply agreement between the organisation and the database. DCCs are not research sites for the purposes of the RGF.

Database managers are advised to provide R&D offices at all DCCs with a copy of the REC application for information, together with a copy of the favourable opinion letter when available. All DCCs should be listed in Part C of the REC application.

NHS researchers undertaking specific research projects using data supplied by a database must apply for permission to R&D offices at all organisations where the research is conducted, whether or not the database has ethical approval.

Site-specific assessment (SSA) is not a requirement for ethical review of research databases.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements
The attached standard conditions give detailed guidance on reporting requirements for research databases with a favourable opinion, including:

- Notifying substantial amendments
- Submitting Annual Progress reports

The HRA website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website.

http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/

HRA Training

We are pleased to welcome researchers and R&D staff at our training days – see details at

http://www.hra.nhs.uk/hra-training/

15/SC/0008 Please quote this number on all correspondence

Yours sincerely

[Signature]

Dr John Sheridan
Chair

E-mail: rrescommittee.southcentral-berkshireb@nhs.net

Enclosures: Approval conditions
### PORTSMOUTH BIRTH COHORT REGISTRY

Wave 0 Questionnaire for Pregnant Women

Unique Identification Number (UIN): ……………………..

#### IDENTIFICATION

<table>
<thead>
<tr>
<th>Name &amp; Address</th>
<th>Date seen</th>
<th>/ /</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Expected date of delivery</td>
<td>/ /</td>
</tr>
<tr>
<td></td>
<td>Is this pregnancy</td>
<td>Single</td>
</tr>
<tr>
<td></td>
<td>Gestational age</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Weight</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Height</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NHS Number</td>
<td></td>
</tr>
<tr>
<td>Hospital Number</td>
<td>Other contact:</td>
<td></td>
</tr>
<tr>
<td>Mother’s date of Birth</td>
<td>Tel No: (Home)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Work)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Mobile)</td>
<td></td>
</tr>
<tr>
<td>Email address:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Consultant</td>
<td>Clinic</td>
<td></td>
</tr>
<tr>
<td>Community Midwife</td>
<td>Surgery</td>
<td></td>
</tr>
</tbody>
</table>

Detach after data collection…………………………………………..
Appendix 5.6. Portsmouth Birth Cohort (PBC) wave 0 questionnaire
PORTSMOUTH BIRTH COHORT STUDY

Wave 0 Questionnaire for Pregnant Women

Unique Identification Number (UIN):…………………………

<table>
<thead>
<tr>
<th>Date seen</th>
<th>/ /</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expected date of delivery</td>
<td>/ /</td>
</tr>
</tbody>
</table>

Is this pregnancy
Single
Multiple

Gestational age

Weight

Height

MODULE 1: SOCIO-ECONOMIC AND DEMOGRAPHIC BACKGROUND

1.1 What is your marital status?

- Domestic partnership
- Married
- Separated
- Divorced
- Widowed
- Single

1.2 With whom do you live?

- Husband/partner
- Parents
- Alone
- Others (specify)

1.3 Is your husband/partner the biological father of your current child? (in pregnancy)

- Yes
- No
- Don’t know

1.3a Did you use any of the following for this pregnancy?

- Donor Egg
- Donor Sperm
- None of these

1.4 Who owns the property in which you live?

- Own home
- Parents’ home
- Housing Association
- Private rent
- Other (specify)

1.5 What is your highest level of education?

- School
- Further (after GCSEs)
- Higher (University)
- Don’t know

1.6 What is your husband/partner’s highest level of education?

- School
- Further (after GCSEs)
1.7 What is your occupation (or usual occupation)?

1.8 What is your husband/partner’s occupation?

1.9 How many children have you had prior to this pregnancy?

<table>
<thead>
<tr>
<th>Sex</th>
<th>Current Age (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1.10 How many children live with you in your house?

1.11 How many are your biological children?

1.12 Have you ever had an induced abortion, miscarriage, stillbirth or infant death?

If yes:
- Number of induced abortions
- Number of spontaneous abortions/miscarriages
- Number of stillbirths
- Number of infant deaths (within 1 year of birth)

1.13 Have any of the following persons ever lived in care?

- You
- Your partner/husband
- Any children (you are the biological parent)
- Child’s father (if different)

1.14 Are any of the following persons currently in care?

- You
- Your partner/husband
Any children (you are the biological parent)  
Yes¹  
No²  
Don’t know³  

Child’s father (if different)  
Yes¹  
No²  
Don’t know³  

1.15 Do you have any pets at home?  
Cat  Yes¹  
No²  
Don’t know³  

Dog  Yes¹  
No²  

Other  Yes¹  
No²  
What?  

MODULE 2: TYPE OF DIET AT HOME

2.1 Which kind of diet best describes your eating habits?  
Normal diet¹  
Vegetarian diet²  
Vegan diet³  
Special diet (specify)⁴  

2.2 Which method best describes your food preparation methods?  
Mainly home cooked food¹  
Mainly commercially prepared foods²  
50/50  

These next questions are about the food eaten in your family. People do different things when they are running out of money for food to make their food or their food money go further.

2.3 In the last 12 months since (date 12 months ago) did you (or other adults in your household) ever cut the size of your meals or skip meals because there wasn’t enough money for food  
Yes¹  
No²  

2.4 If yes, How often did this happen?  
Almost every month¹  
Some months but not every month²  
Only in 1 or 2 months³  

2.5 In the last 12 months did you ever eat less than you felt you should because there wasn’t enough money to buy food?  
Yes¹  
No²  

2.6 In the last 12 months since (date 12 months ago) were you ever hungry but didn’t eat because you couldn’t afford food?  
Yes¹  
No²  

Now I’m going to read you 2 statements that people have made about their food situation. For these statements please tell me whether the statement was often, sometimes or never true for you (or the other members of your household) in the last 12 months.

2.7. The first statement is ‘The food that I bought just didn’t last and I didn’t have enough money to get more.’  
Often¹  
Sometimes²  
Never true³  

2.8 ‘I couldn’t afford to eat balanced meals’  
Often¹  
Sometimes²  
Never true³  

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MODULE 3: SMOKING / DRINKING HABITS OF PREGNANT WOMEN

3.1 Do you normally smoke?  
Yes  
No  
If No go to Q 3.3

3.2 If yes:  
Have you stopped smoking during this pregnancy?  
Yes  
No
  Have you cut down during this pregnancy?  
Yes  
No
  How many cigarettes do you smoke daily on average?  

3.3 Do other people in the house smoke?  
Yes  
No
If No go to Q 3.3

3.4 If yes, who in the household smokes?  
Husband/partner  
Parents  
Children (you are the biological parent)  
Others

3.5 Do they smoke inside the house?  
Yes  
No

3.6 When you are not pregnant, do you normally consume alcohol?  
Yes  
No
If No go to Q. 4

3.7 If yes, how much do you normally drink each week, when you are not pregnant?  
Less than 1 unit  
2-10 units  
11-21 units  
22-35 units  
36-50 units  
51 units and over

3.8 Have you stopped drinking during this pregnancy?  
Yes  
No
If yes, go to Q.4

3.9 If no, have you cut down alcohol consumption during this pregnancy?  
Yes  
No

3.10 How often do you drink?  
Almost every day  
5-6 days a week  
3-4 days a week  
Once or twice a week  
Once or twice a month  
Once every couple of months

3.11 How many units of alcohol do you currently drink each week?  
Less than 1 unit  
2-10 units  
11-21 units  
22-35 units  
36-50 units  
51 units and over

MODULE 4: FAMILY HISTORY OF MENTAL HEALTH

4.1 Have you or any of the following people in your family ever been diagnosed with clinical depression?  
You  
Yes  
No  
Don’t know

Your partner/husband  
Yes  
No  
Don’t know

Any children (you are the biological parent)  
Yes  
No
4.2 Do you or any of the following people in your family currently suffer with clinical depression?

**You**
- Yes\(^1\)
- No\(^2\)
- Don’t know\(^3\)

**Your partner/husband**
- Yes\(^1\)
- No\(^2\)
- Don’t know\(^3\)

**Any children (you are the biological parent)**
- Yes\(^1\)
- No\(^2\)
- Don’t know\(^3\)

4.3 Have you or any of the following people in your family ever had a diagnosis of anxiety?

**You**
- Yes\(^1\)
- No\(^2\)
- Don’t know\(^3\)

**Your partner/husband**
- Yes\(^1\)
- No\(^2\)
- Don’t know\(^3\)

**Any children (you are the biological parent)**
- Yes\(^1\)
- No\(^2\)
- Don’t know\(^3\)

4.4 Do you or any of the following people in your family currently suffer with anxiety?

**You**
- Yes\(^1\)
- No\(^2\)
- Don’t know\(^3\)

**Your partner/husband**
- Yes\(^1\)
- No\(^2\)
- Don’t know\(^3\)

**Any children (you are the biological parent)**
- Yes\(^1\)
- No\(^2\)
- Don’t know\(^3\)

4.5 Have any of the following persons ever been diagnosed with learning difficulties e.g. dyslexia?

**You**
- Yes\(^1\)
- No\(^2\)
- Don’t know\(^3\)

**Your partner/husband**
- Yes\(^1\)
- No\(^2\)
- Don’t know\(^3\)

**Any children (you are the biological parent)**
- Yes\(^1\)
- No\(^2\)
- Don’t know\(^3\)

4.6 Have any of the following persons ever been diagnosed with autism?

**You**
- Yes\(^1\)
- No\(^2\)
- Don’t know\(^3\)
### MODULE 5: FAMILY HISTORY OF PHYSICAL HEALTH

#### 5.1 Have you or any of the following persons ever had Cardio-Vascular Disease?

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
<th>Don’t know</th>
</tr>
</thead>
<tbody>
<tr>
<td>You</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Your partner/husband</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any children (you are the biological parent)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### 5.2 Have you or any of the following persons ever had Diabetes Mellitus?

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
<th>Don’t know</th>
</tr>
</thead>
<tbody>
<tr>
<td>You</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Your partner/husband</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any children (you are the biological parent)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### 5.3 Have you or any of the following persons ever had Epilepsy?

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
<th>Don’t know</th>
</tr>
</thead>
<tbody>
<tr>
<td>You</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Your partner/husband</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any children (you are the biological parent)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### 5.4 Have you or any of the following persons ever had High blood pressure?

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
<th>Don’t know</th>
</tr>
</thead>
<tbody>
<tr>
<td>You</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Your partner/husband</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Question</th>
<th>(You)</th>
<th>(Your partner/husband)</th>
<th>(Any children)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.5 Have any of the following persons ever suffered from cancer?</td>
<td>Yes¹</td>
<td>Yes¹</td>
<td>Yes¹</td>
</tr>
<tr>
<td></td>
<td>No²</td>
<td>No²</td>
<td>No²</td>
</tr>
<tr>
<td></td>
<td>Don’t know³</td>
<td>Don’t know³</td>
<td>Don’t know³</td>
</tr>
<tr>
<td>5.6 Have any of the following persons ever been diagnosed with asthma?</td>
<td>Yes¹</td>
<td>Yes¹</td>
<td>Yes¹</td>
</tr>
<tr>
<td></td>
<td>No²</td>
<td>No²</td>
<td>No²</td>
</tr>
<tr>
<td></td>
<td>Don’t know³</td>
<td>Don’t know³</td>
<td>Don’t know³</td>
</tr>
<tr>
<td>5.7 Have any of the following persons ever been diagnosed with hay fever?</td>
<td>Yes¹</td>
<td>Yes¹</td>
<td>Yes¹</td>
</tr>
<tr>
<td></td>
<td>No²</td>
<td>No²</td>
<td>No²</td>
</tr>
<tr>
<td></td>
<td>Don’t know³</td>
<td>Don’t know³</td>
<td>Don’t know³</td>
</tr>
<tr>
<td>5.8 Have any of the following ever had an itchy rash which was coming and going for at least six months?</td>
<td>Yes¹</td>
<td>Yes¹</td>
<td>Yes¹</td>
</tr>
<tr>
<td></td>
<td>No²</td>
<td>No²</td>
<td>No²</td>
</tr>
<tr>
<td></td>
<td>Don’t know³</td>
<td>Don’t know³</td>
<td>Don’t know³</td>
</tr>
</tbody>
</table>
Any children (you are the biological parent)  Yes\(^1\)  No\(^2\)  Don’t know\(^3\)

5.9 Have any of the following persons ever had wheezing or whistling in the chest at any time in the past?

You  Yes\(^1\)  No\(^2\)  Don’t know\(^3\)

Your partner/husband  Yes\(^1\)  No\(^2\)  Don’t know\(^3\)

Any children (you are the biological parent)  Yes\(^1\)  No\(^2\)  Don’t know\(^3\)

5.10 Have any of the following persons ever suffered from an itchy, stuffy or runny nose and/or swollen, itchy eyes when they did not have a cold?

You  Yes\(^1\)  No\(^2\)  Don’t know\(^3\)

Your partner/husband  Yes\(^1\)  No\(^2\)  Don’t know\(^3\)

Any children (you are the biological parent)  Yes\(^1\)  No\(^2\)  Don’t know\(^3\)

5.11 Have any of the following persons ever suffered from food allergy or intolerance at any time in the past?

You  Yes\(^1\)  No\(^2\)  Don’t know\(^3\)

Your partner/husband  Yes\(^1\)  No\(^2\)  Don’t know\(^3\)

Any children (you are the biological parent)  Yes\(^1\)  No\(^2\)  Don’t know\(^3\)

5.12 If yes, what food/s?

<table>
<thead>
<tr>
<th></th>
<th>Food 1</th>
<th>Food 2</th>
<th>Food 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>You</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Your partner/husband</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Child 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Child 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Child 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Child 4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
MODULE 6: PHYSICAL EXERCISE

6.1 Do you usually take regular exercise (including brisk walking - more than 15 minutes at a time)?

Yes 1  No 2

6.2 If yes what sort of exercise (write all exercise that is undertaken each week)


MODULE 7: MEDICATION

7.1 Are you currently taking any medications?

Yes 1  No 2

7.2 If yes, please specify all


MODULE 8: ETHNICITY AND RELIGION

8.1 What is your religion?

Christian 1 (including Church of England, Catholic, Protestant and all other Christian denominations)

No religion 2  Buddhist 3  Hindu 4  Jewish 5  Muslim 6  Sikh 7

Any other religion, please describe 8

8.2 What is your ethnic group?

White 1  English/Welsh/Scottish/Northern

Irish/British

Irish

Gypsy or Irish Traveller

Any other White background, please describe

Mixed/Multiple ethnic groups 5

White and Black Caribbean

White and Black African

White and Asian

Any other Mixed/Multiple ethnic background, please describe

Asian/Asian British 3

Indian

Pakistani

Bangladeshi

Chinese

Any other Asian background, please describe

Black/African/Caribbean/Black British 4

African

Caribbean

Any other Black/African/Caribbean background, please describe
MODULE 9: GENERAL

9.1 What language do you normally speak at home? Please specify all:

Language normally spoken in the home:

Other languages spoken in the home:

9.2 OTHER COMMENTS

Other ethnic group:
Arab
Any other ethnic group, please describe:

Appendix 5.7. Portsmouth Birth Cohort (PBC) birth questionnaire (wave 1)

Portsmouth Birth Cohort Registry
Wave 1

Unique Identification Number (UIN):
(MULTIPLE BIRTHS WILL SHARE SAME IDENTIFICATION NUMBER WITH A
SUPERSCRIPT OF 1,2..Etc)

<table>
<thead>
<tr>
<th>IDENTIFICATION</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother’s Name:</td>
<td></td>
</tr>
<tr>
<td>Mother’s Address:</td>
<td></td>
</tr>
<tr>
<td>Postcode:</td>
<td></td>
</tr>
<tr>
<td>Mother’s Hospital Number:</td>
<td></td>
</tr>
<tr>
<td>Child’s Hospital Number:</td>
<td></td>
</tr>
</tbody>
</table>

Detach after data collection……………………………………..
Appendix 5.7. Portsmouth Birth Cohort (PBC) birth questionnaire (wave 1)

Portsmouth Birth Cohort Study
Wave 1

Unique Identification Number (UIN):
To be collected soon after discharge from the hospital
(MULTIPLE BIRTHS WILL SHARE SAME IDENTIFICATION NUMBER
WITH A SUPERScript OF 1,2… Etc)

1. DELIVERY AND RELATED INFORMATION

<table>
<thead>
<tr>
<th>Pregnancy outcome</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Live birth(^1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spontaneous abortion(^2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Induced abortion(^3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Still birth(^4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Twins/multiples</td>
<td></td>
<td>Yes(^1)</td>
<td>No(^2)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Date of Delivery</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Child’s sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male(^1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female(^2)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Child’s DOB             |     |     |     |
| Birth weight            |     |     |     |
| kg                      |     |     |     |
| lb                      |     |     |     |
| oz                      |     |     |     |

| Type of delivery        |     |     |     |
| Normal\(^1\)            |     |     |     |
| Caesarian\(^2\)         |     |     |     |
| Instrumental\(^3\)      |     |     |     |

| Perineal trauma or Episiotomy |     |     |     |
| 1\(^{st}\) degree           |     |     |     |
| 2\(^{nd}\) degree           |     |     |     |
| 3\(^{rd}\) degree           |     |     |     |
| Episiotomy                  |     |     |     |
| None                        |     |     |     |

| Estimated blood loss at delivery (mls) |     |     |     |

| Method of feeding on the day of birth |     |     |     |
| Breast\(^1\)                         |     |     |     |
| Bottle\(^2\)                         |     |     |     |
| Both\(^3\)                           |     |     |     |

| Apgars at birth |     |     |     |
| 1 minute        |     |     | N/A\(^{-100}\) |
| 5 minutes       |     |     |     |

| Problems |     |     |     |

| Admitted to NICU |     |     |     |
| Yes\(^1\)        |     | No\(^2\) | D/K\(^3\) |

| Number of days spent at QA after delivery? |     |     |     |
| Was it more than expected number of days? |     |     |     |
| Yes\(^1\)                         |     | No\(^2\) | D/K\(^3\) |

| If more than normal, why? Was there any postnatal complications? |     |     |     |

2. ANY OTHER COMMENTS

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Appendix 5.8. Ethical approval for the PBC revised questionnaires from Berkshire NHS

12 May 2015

Professor Taraneh (Tara) Dean
University of Portsmouth
Purple Door, 28 Guildhall Walk
Portsmouth PO1 2DD

Dear Professor Dean

Title of the Database: Portsmouth Birth Cohort Registry
REC reference: 15/SC/0008
Amendment number: Substantial amendment dated 06 May 2015
Amendment date: 06 May 2015
IRAS project ID: 162850

The above amendment was reviewed by the Sub-Committee in correspondence.

Ethical opinion

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

<table>
<thead>
<tr>
<th>Document</th>
<th>Version</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Covering letter on headed paper [NoSA_15-04-30_coverletter final]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Notice of Substantial Amendment (RD) [AmendmentFormResearchDatabase_snapshot]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other [FFQ in Pregnancy logo amend GG]</td>
<td>2</td>
<td>05 August 2005</td>
</tr>
<tr>
<td>Other [Milk diary-1 month TD logo amend GG]</td>
<td>2</td>
<td>05 August 2005</td>
</tr>
<tr>
<td>Other [Milk diary-4 month TD logo amend GG]</td>
<td>2</td>
<td>05 August 2005</td>
</tr>
</tbody>
</table>

Membership of the Committee

The members of the Ethics Committee who took part in the review are listed on the attached

A Research Ethics Committee established by the Health Research Authority
sheet.
None.

**Statement of compliance**

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

We are pleased to welcome researchers and R & D staff at our NRES committee members’ training days – see details at [http://www.hra.nhs.uk/hra-training/](http://www.hra.nhs.uk/hra-training/)

| 15/SC/0008 | Please quote this number on all correspondence |

Yours sincerely

Mr Wai Yeung  
Research Ethics Committee (REC) Assistant

pp Dr John Sheridan – Chair of South Central - Berkshire B REC

E-mail: nrescommittee.southcentral-berkshireb@nhs.net

**Enclosures:** List of names and professions of members who took part in the review

**Copy to:** Mrs Samantha Hill, University of Portsmouth  
Confidentiality Advice Team

A Research Ethics Committee established by the Health Research Authority
NRES Committee South Central - Berkshire B

Attendance at Sub-Committee of the REC meeting in correspondence

**Committee Members:**

<table>
<thead>
<tr>
<th>Name</th>
<th>Profession</th>
<th>Present</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mr Mike Amott</td>
<td>Research Consultant</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Mrs Rachel Quinn</td>
<td>Nurse Member</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Dr John Sheridan</td>
<td>Consultant Toxicologist and Chemist</td>
<td>Yes</td>
<td></td>
</tr>
</tbody>
</table>

**Also in attendance:**

<table>
<thead>
<tr>
<th>Name</th>
<th>Position (or reason for attending)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mr Wai Yeung</td>
<td>REC Assistant</td>
</tr>
</tbody>
</table>
Appendix 5.9. R&D approval letter for the PBC registry from Portsmouth Hospitals, NHS Trust

Portsmouth Hospitals  
NHS Trust

Research & Development Department  
1st Floor, Gloucester House  
Queen Alexandra Hospital  
Cosham  
Portsmouth  
PO6 3LY

Tel: 023 9228 6236  
Fax: 023 9228 6037

Web: www.porthosp.nhs.uk

Hannah Doe  
Consultant Midwife  
Maternity Department  
Queen Alexandra Hospital  
Cosham  
Portsmouth PO6 3LY

11th December 2015 – reissued 21st December 2015 to include protocol V6.0

Dear Hannah

Re: NHS Organisational Permission – Non CTIMP research

Study Title: Portsmouth Birth Cohort Registry

Research Office No: PHT/2015/112  Sponsor: University of Portsmouth

Chief Investigator: Professor Taraneh Dean, Purple Door, 28 Guildhall Walk, University of Portsmouth, PO1 2DD

I have received confirmation that the above study has been processed through the Portsmouth Research Office. The Office has reviewed your submission and confirms that it meets the requirements of the Trust and Research Governance Framework.

On behalf of Portsmouth Hospitals NHS Trust I therefore give NHS organisational permission for the above named project to commence.

Conditions of Approval

1. That you accept the responsibility of Principal Investigator as defined in the current Research Governance Framework and as you have declared in your signed SSIF. (http://www.dh.gov.uk/en/Aboutus/Researchanddevelopment)

2. Submit any amendments to the study documentation to the Research Office.

3. Ensure all study personnel, not employed by Portsmouth Hospitals NHS Trust, hold either honorary research contracts/letters of access with this Trust, before they have access to any facilities, patients, staff, their data, tissue or organs.

Non-CTIMP Approval Letter – V2.0 20/01/2015  
Page 1 of 3
4. Where required, submit copies of Serious Adverse Events, SSAR’s and SUSAR’s involving subjects from this Trust to the Sponsor immediately in line with PHT/RDSOP/007. All locally occurring SSAR’s, SUSAR’s and safety concerns must also be reported to the Research Office. Ensure that the SAE Alert Notice is included in all versions of the health record for the SAE reporting period, where applicable.

5. Report all USMS and Serious Breaches immediately in line with PHT/RDSOP/002 and PHT/RDSOP/006.

6. Complete GCP Compliance Tool returns as requested.

7. Maintain an Investigator Site File (ISF) within your department containing essential study documentation in accordance with PHT/RDSOP/009 and ensure that all members of the research team are aware of and understand their responsibilities under the Research Governance Framework for Health and Social Care. Your ISF must be available at all times for monitoring purposes and you must inform the Research Office of the ISF location at commencement of the project and returning by post or e-mail to: research.office@porthosp.nhs.uk. A template for the ISF is available for your use; please refer to PHT/RDSOP/009.

8. Enter recruitment data onto the Portsmouth Hospitals EDGE database in accordance with local research governance. If you do not have access to EDGE, please contact the Research Office; access and training will be arranged.

9. You will use the enclosed stickers in accordance with the guidance included with this letter.

10. Agree to conduct this research project in accordance with the conditions of this approval and all subsequent SOPs. For all recent documents, please refer to http://www.porthosp.nhs.uk/Research-Department/policies-sops.htm.

Additional conditions of approval:

11. Sponsor condition: It is requirement that all future projects that are hosted by the Trust and linked to the Portsmouth Birth Cohort are adopted on to the NIHR Portfolio prior to receiving NHS Organisational Permission.

12. Please send the CV and GCP certificates for Hannah Doe and Linda Lishman before recruitment starts.

Please ensure we are copied in to all correspondence and reporting requirements of the National Research Ethics Service (NRES). This includes annual reports submitted by Chief Investigator and the end of study declaration. We should also be informed of any publications or conference presentations resulting from this research.

Should you find yourself unsure of any of the above requirements please do not hesitate to contact the Research Office for support.

Documents submitted are detailed below.

<table>
<thead>
<tr>
<th>Document Name</th>
<th>Version</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>IRAS Application</td>
<td>Full Dataset (DRAFT)</td>
<td>-</td>
</tr>
<tr>
<td>Patient Information</td>
<td>V4.0</td>
<td>28 Oct 2015</td>
</tr>
<tr>
<td>Patient Consent Form</td>
<td>V3.0</td>
<td>28 Oct 2015</td>
</tr>
<tr>
<td>Questionnaire: Baby Eating Behaviour</td>
<td>V1.0</td>
<td>30 Oct 2015</td>
</tr>
<tr>
<td>--------------------------------------</td>
<td>------</td>
<td>-------------</td>
</tr>
<tr>
<td>Questionnaire: Wave 0</td>
<td>V3.0</td>
<td>30 Oct 2015</td>
</tr>
<tr>
<td>Questionnaire: Wave 1</td>
<td>V3.0</td>
<td>28 Oct 2015</td>
</tr>
<tr>
<td>Questionnaire: FFQ in Pregnancy</td>
<td>V3.0</td>
<td>30 Oct 2015</td>
</tr>
<tr>
<td>Milk Diary 1 Month</td>
<td>V2.0</td>
<td>05 Aug 2005</td>
</tr>
<tr>
<td>Milk Diary 4 Month</td>
<td>V2.0</td>
<td>05 Aug 2005</td>
</tr>
<tr>
<td>REC approval letter 5/SC/0008</td>
<td>23 Jan 2015</td>
<td></td>
</tr>
<tr>
<td>REC approval letter 5/SC/0008 (Amendment 2)</td>
<td>11 Nov 2015</td>
<td></td>
</tr>
<tr>
<td>REC approval letter 5/SC/0008 (Amendment 3)</td>
<td>09 Dec 2015</td>
<td></td>
</tr>
<tr>
<td>Protocol</td>
<td>V6.0</td>
<td>12 Nov 2015</td>
</tr>
</tbody>
</table>

Yours sincerely

Helen Munday
Associate Research Manager

Cc: CI; Professor T Dean; Research Fellows: Dr Suzannah Helps, Dr Kate Maslin; Research Midwives: Zoe Garner, Jess Harris, Linda Lishman; Trust Lead Research Nurses: Sharon McCreedy, Johanna Mouland.

Enc. Investigator Site File Location Form
Clinical Trial Identification Stickers
Guidance on the Use of Research Stickers
Appendix 5.10. Ethical approval letter for amendments requested for the FFQ-P

28 January 2016

Professor Taranah (Tara) Dean
University of Portsmouth
Purple Door, 28 Guildhall Walk
Portsmouth
PO1 2DD

Dear Professor Dean

Title of the Database: Portsmouth Birth Cohort Registry
REC reference: 15/SC/0008
Amendment number: 5:22/01/16 - Food Frequency Questionnaire updated
Amendment date: 22 January 2016
IRAS project ID: 162850

The above amendment was reviewed at the meeting of the Sub-Committee held on 28th January 2016 by the Sub-Committee in correspondence.

Ethical opinion

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

<table>
<thead>
<tr>
<th>Document</th>
<th>Version</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Covering letter on headed paper [Coverletter220116]</td>
<td></td>
<td>22 January 2016</td>
</tr>
<tr>
<td>Notice of Substantial Amendment (RD) [AmendmentForm:ResearchDatabase_ReadyForSubmission (4)]</td>
<td></td>
<td>22 January 2016</td>
</tr>
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<td>Other [FFQ in Pregnancy Version 4 220116 Tracked changes]</td>
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<td>22 January 2016</td>
</tr>
<tr>
<td>Other [FFQ in Pregnancy Version 4 220116 Clean]</td>
<td>4</td>
<td>22 January 2016</td>
</tr>
</tbody>
</table>

Membership of the Committee

The members of the Ethics Committee who took part in the review are listed on the
attached sheet.

**Statement of compliance**

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

We are pleased to welcome researchers and R&D staff at our NRES committee members' training days – see details at [http://www.hra.nhs.uk/hra-training/](http://www.hra.nhs.uk/hra-training/)

| 15/SC/0008 | Please quote this number on all correspondence |

Yours sincerely

PP

**Dr John Sheridan**
**Chair**

E-mail: nrescommittee.southcentral-berkshireb@nhs.net

**Enclosures:** List of names and professions of members who took part in the review

**Copy to:** Mrs Samantha Hill, University of Portsmouth  Confidentiality Advice Team
South Central - Berkshire B Research Ethics Committee

Attendance at Sub-Committee of the REC meeting on 01 February 2016

Committee Members:

<table>
<thead>
<tr>
<th>Name</th>
<th>Profession</th>
<th>Present</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mr Mike Arnott</td>
<td>Research Consultant</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Dr John Sheridan (Chan)</td>
<td>Consultant Toxicologist and Chemist</td>
<td>Yes</td>
<td></td>
</tr>
</tbody>
</table>

Also in attendance:

<table>
<thead>
<tr>
<th>Name</th>
<th>Position (or reason for attending)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miss Tina Cavalliere</td>
<td>REC Manager</td>
</tr>
</tbody>
</table>
Appendix 5.11. Ethical approval letter for amendments requested for the MD questionnaire

Health Research Authority

South Central - Berkshire B Research Ethics Committee
Whitefriars
Level 3, Block B
Levens Mead
Bristol
BS1 2NT

Telephone: 0207 104 8037

09 May 2016

Professor Taraneh (Tara) Dean
University of Portsmouth
Purple Door, 28 Guildhall Walk
Portsmouth
PO1 2DD

Dear Professor Dean

Title of the Database: Portsmouth Birth Cohort Registry
REC reference: 15/SC/0008
Amendment number: Amendment 7; 27/04/2016
Amendment date: 27 April 2016
IRAS project ID: 162850

The above amendment was reviewed at the meeting of the Sub-Committee held on 9th May 2016 by the Sub-Committee in correspondence.

Ethical opinion

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

<table>
<thead>
<tr>
<th>Document</th>
<th>Version</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Covering letter on headed paper [Coverletter270416]</td>
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<td></td>
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<tr>
<td>Notice of Substantial Amendment (RD) [AmendmentFormResearchDatabase_ReadyForSubmission (7)]</td>
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<td>27 April 2016</td>
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<td>27 April 2016</td>
</tr>
</tbody>
</table>

Membership of the Committee

The members of the Ethics Committee who took part in the review are listed on the
attached sheet.

**Statement of compliance**

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

We are pleased to welcome researchers and R & D staff at our NRES committee members’ training days – see details at [http://www.hra.nhs.uk/hra-training/](http://www.hra.nhs.uk/hra-training/)

15/SC/0008 Please quote this number on all correspondence

Yours sincerely

Mr Mike Arnott
Vice Chair

E-mail: nrescommittee.southcentral-berkshireb@nhs.net

**Enclosures:** List of names and professions of members who took part in the review

**Copy to:** Mrs Samantha Hill, University of Portsmouth
Confidentiality Advice Team
South Central - Berkshire B Research Ethics Committee

Attendance at Sub-Committee of the REC meeting on 9 May 2016

Committee Members:

<table>
<thead>
<tr>
<th>Name</th>
<th>Profession</th>
<th>Present</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mr Mike Arnott (Vice Chair)</td>
<td>Research Consultant</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Mr John Inman</td>
<td>Pharmacist (retired)</td>
<td>Yes</td>
<td></td>
</tr>
</tbody>
</table>

Also in attendance:

<table>
<thead>
<tr>
<th>Name</th>
<th>Position (or reason for attending)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ms Helen Sivey</td>
<td>REC Assistant</td>
</tr>
</tbody>
</table>
Appendix 5.12. Ethical approval letter for the 6 months follow-up questionnaire

08 July 2016

Professor Taranah (Tara) Dean
University of Portsmouth
Purple Door, 28 Guildhall Walk
Portsmouth
PO1 2DD

Dear Professor Dean

Title of the Database: Portsmouth Birth Cohort Registry
REC reference: 15/SC/0008
Amendment number: 8
Amendment date: 17 June 2016
IRAS project ID: 162850

The above amendment was reviewed at the meeting of the Sub-Committee held on 08 July 2016 via correspondence.

Ethical opinion

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

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<th>Document</th>
<th>Version</th>
<th>Date</th>
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<tr>
<td>Notice of Substantial Amendment (RD) [AmendmentFormResearchDatabase_snapshot (3)]</td>
<td>8</td>
<td>17 June 2016</td>
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<tr>
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<td>17 June 2016</td>
</tr>
</tbody>
</table>

Membership of the Committee

The members of the Ethics Committee who took part in the review are listed on the attached sheet.

None
Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

We are pleased to welcome researchers and R & D staff at our NRES committee members’ training days – see details at http://www.hra.nhs.uk/hra-training/

15/SC/0098 Please quote this number on all correspondence

Yours sincerely

[Signature]

Ms Helen Sivey
REC Assistant

pp. Dr John Sheridan
Chair

E-mail: nrescommittee.southcentral-berkshireb@nhs.net
South Central - Berkshire B Research Ethics Committee
Attendance at Sub-Committee of the REC meeting on 08 July 2016

Committee Members:

<table>
<thead>
<tr>
<th>Name</th>
<th>Profession</th>
<th>Present</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr Mike Proven</td>
<td>Coordinator for QAR (UREC Secretary)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dr John Sheridan [Chair]</td>
<td>Consultant Toxicologist and Chemist</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Also in attendance:

<table>
<thead>
<tr>
<th>Name</th>
<th>Position (or reason for attending)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ms Helen Slivey</td>
<td>REC Assistant</td>
</tr>
</tbody>
</table>
Appendix 6.1. Infant’s food frequency questionnaire (FFQ) at 6 months

Portsmouth Birth Cohort Study

Six Month Questionnaire
Instructions for completion

Dear parent

You have received this questionnaire as part of the Portsmouth Birth Cohort Study.

Now that your baby is six months old we are interested in finding out about how he/she is growing.

In this questionnaire, we will ask you about:

- Your baby’s health
- The kinds of foods that your baby is eating
- How well your baby sleeps
- Your baby’s behaviour

This questionnaire will take about 30 minutes to complete.

We would really appreciate it if you can answer all of the questions.

Please send back the completed form using the enclosed pre-paid addressed envelope. If you have any queries, please do contact me in relation to my role as Principal Investigator for this study.

Yours faithfully

Professor Tara Dean
Dean of Science
Date Questionnaire is completed

Who completed the questionnaire?
- Mother
- Father
- Grandparent
- Other

Who?

1.1 What is your baby’s current or most recent weight (please look in your red book)?

Weight: 
Date weighed:

1.2 What is your baby’s length (if known)?

Length: 
Date measured:

2 YOUR BABY’S HEALTH

We would like to know about your baby’s health.
Has your baby experienced any of the following since they were born?

2.1 Has your baby ever had wheezing or whistling in the chest?

Yes  No  Don’t know

2.2 Has your baby had a dry cough at night, apart from the cough associated with a cold or a chest infection?

Yes  No  Don’t know

2.3 Has your baby ever had an itchy rash that was coming and going?
If No or Don’t know, go to Q 2.4

Yes  No  Don’t know

2.3.1 If yes, where on their body does your baby get the itchy rash?

2.3.2 Have you identified the cause of the itchy rash?

Yes  No  Don’t know

2.3.3 If yes, what?

Food  Yes  No  Please specify:
Animals  Yes  No
House dust mite  Yes  No
Other  Yes  No  Please specify:
2.4 Has your child ever suffered from vomiting (> 1 tbsp)?
If No or Don’t know go to Q2.5

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
<th>Don’t know</th>
</tr>
</thead>
</table>

2.4.1 Have you identified the cause of the vomiting?
If No or Don’t know go to Q2.5

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
<th>Don’t know</th>
</tr>
</thead>
</table>

2.4.2 If yes, what?

<table>
<thead>
<tr>
<th>Food</th>
<th>Yes</th>
<th>No</th>
<th>Please specify:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Other</td>
<td>Yes</td>
<td>No</td>
<td>Please specify:</td>
</tr>
</tbody>
</table>

2.5 Has your baby ever suffered from diarrhoea?
If No or Don’t know go to Q2.6

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
<th>Don’t know</th>
</tr>
</thead>
</table>

2.5.1 Have you identified the cause of the diarrhoea?
If No or Don’t know go to Q2.6

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
<th>Don’t know</th>
</tr>
</thead>
</table>

2.5.2 If yes, what?

<table>
<thead>
<tr>
<th>Food</th>
<th>Yes</th>
<th>No</th>
<th>Please specify:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Other</td>
<td>Yes</td>
<td>No</td>
<td>Please specify:</td>
</tr>
</tbody>
</table>

2.6 Has your baby ever suffered from constipation?
If No or Don’t know go to Q2.7

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
<th>Don’t know</th>
</tr>
</thead>
</table>

2.6.1 Have you identified the cause of the constipation?
If No or Don’t know go to Q2.7

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
<th>Don’t know</th>
</tr>
</thead>
</table>

2.6.2 If yes, what?

<table>
<thead>
<tr>
<th>Food</th>
<th>Yes</th>
<th>No</th>
<th>Please specify:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Other</td>
<td>Yes</td>
<td>No</td>
<td>Please specify:</td>
</tr>
</tbody>
</table>

2.7 Has your baby ever suffered from colic/tummy ache?
If No or Don’t know go to Q2.8

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
<th>Don’t know</th>
</tr>
</thead>
</table>

2.7.1 Have you identified the cause of the colic/tummy ache?
If No or Don’t know go to Q2.8

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
<th>Don’t know</th>
</tr>
</thead>
</table>

2.7.2 If yes, what?

<table>
<thead>
<tr>
<th>Food</th>
<th>Yes</th>
<th>No</th>
<th>Please specify:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Other</td>
<td>Yes</td>
<td>No</td>
<td>Please specify:</td>
</tr>
</tbody>
</table>
2.8 Has your baby ever suffered from any other food related problem?
If No or Don’t know go to Q3

Yes ☐ No ☐ Don’t know ☐

2.8.1 If yes, what was the problem and did you identify the cause?

<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause if identified</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Did you consult your GP or paediatrician about any the symptoms we have asked you about?
If yes, which symptoms did you speak to your GP about and what did the GP recommend?
If no, please go to Q2.9

2.9 Have any of your baby’s teeth started to come through?

Yes ☐ No ☐ Don’t know ☐

2.9.1 If yes, when did your baby’s first tooth come through?
If no, go to Q3

Months

2.9.2 If yes, do you brush your baby’s teeth?

Yes ☐ No ☐ Don’t know ☐

3 YOUR BABY’S FEEDING

3.1 What milk are you feeding your baby?

Exclusively breast milk ☐
Exclusively formula ☐
Mix of breast milk and formula ☐

3.2 If your baby drinks formula milk, how old was he/she when you first introduced formula?
If your baby doesn’t have formula please go to Q 3.3

3.2.1 If your baby has formula, what type do they drink e.g. Aptamil, SMA, hungry baby milk, follow on milk, specialised infant formula?

3.2.2 What does your baby have their formula milk from?

Bottle
3.3 When did you first introduce solids into your baby’s diet?  
If you have not introduced solids please go to Q3.18

<table>
<thead>
<tr>
<th>Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

3.4 Which three foods did you introduce to your baby’s diet first?

<table>
<thead>
<tr>
<th>Food 1</th>
<th>Food 2</th>
<th>Food 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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</tbody>
</table>

3.5 Did you first feed your baby pureed food or finger food (baby-led weaning)?

<table>
<thead>
<tr>
<th>Pureed</th>
<th>Finger food</th>
<th>Mixed</th>
<th>Don’t know</th>
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<tbody>
<tr>
<td></td>
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</tbody>
</table>

3.6 Have you given your baby any of these foods, and if so when did you first give it to them?

<table>
<thead>
<tr>
<th>Food</th>
<th>Never</th>
<th>&lt; 3 months</th>
<th>3-6 months</th>
<th>6-9 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat: e.g. baby rusk, cereals, pasta, bread, cakes, biscuits</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egg: e.g. cooked egg, cakes, brioché, jaffa cakes, quiche</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk: e.g. yoghurt, fromage frais, custard, ice cream, butter, cheese, cow’s milk in foods</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fish: e.g. fish fingers, fish cakes, fish pie</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nuts: e.g. peanut butter, peanut cookies, crunchy nut cornflakes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sesame: e.g. humous, tahini, seed rolls, cereal bars</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

3.7 Are you avoiding any foods from your baby’s diet because of allergy?  

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>
If yes, which foods?

3.8 Do you mainly feed your baby homemade or shop bought baby foods?

Homemade
Shop bought
50/50

3.9 Does your baby have any medical conditions that affects what you feed them?

Yes ☐ No ☐ Don’t know ☐

If yes, please specify:

3.10 Does your baby currently take any dietary supplements (e.g. vitamins)

Yes ☐ No ☐ Don’t know ☐

If yes, please specify:

3.11 How much attention is paid to your baby’s diet in terms of healthy eating?

Very little ☐ Some what ☐ A great deal ☐

3.12 Where did you get advice about weaning (which foods and when to feed) your baby?

Please tick ALL the ones that apply

Health visitor ☐ Internet ☐ Books ☐ Mother ☐ Grandmother ☐ Friends with same age children ☐ Friends with older children ☐ Leaflet ☐ GP or other medical professional ☐

Other, please state ☐
3.13 Which ONE source of advice about weaning (which foods and when to feed your baby) did you find the most influential? Please tick ONE

Health visitor
Internet
Books
Mother
Grandmother
Friends with same age children
Friends with older children
Leaflet
GP or other medical professional
Other, please state

3.14 Thinking about the advice you were given on what and when to feed your baby. How consistent (giving the same advice) was the information from different sources e.g. health visitor, internet, books, family and friends.

Please tick on the scale below

<table>
<thead>
<tr>
<th>Very consistent</th>
<th>Very conflicting</th>
</tr>
</thead>
<tbody>
<tr>
<td>they gave the same advice</td>
<td>they gave very different advice</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

3.15 How confident are you deciding which foods and when to feed your baby?

Please tick on the scale below

<table>
<thead>
<tr>
<th>Very confident</th>
<th>Not at all confident</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>
We would like to know about the foods your baby has eaten in the past month. We have listed various foods many of which may not have been eaten in the past month or ever.

You should only include food actually eaten, do not include food that was left over or spilled.

Thinking about the PAST MONTH please tick to say how often your baby has eaten each food.

<table>
<thead>
<tr>
<th></th>
<th>Never</th>
<th>1-3 per month</th>
<th>Number of times per week</th>
<th>More than once a day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

**READY MADE BABY FOODS**

- Pure baby rice (not including fruit flavoured rice)
- Other dried baby cereals
- Rusks
- Dried meat or fish based meals
- Dried vegetable, pasta or rice based meals
- Dried desserts
- Ready-made breakfast meals (e.g. porridge)
- Ready-made meat or fish based meals
- Ready-made vegetable, pasta or rice based savoury meals
- Ready-made milk of cereal based deserts
- Ready-made pure fruit puree
- Other ready-made fruit based desserts (not including pure fruit puree)

**OTHER FOODS**

- Weetabix or other wheat biscuits
- Other cereals, not including Weetabix or baby cereals
- Potatoes
- Rice
- Pasta including tinned spaghetti
- Meat
- Fish
- Beans and pulses, including baked beans, kidney beans, chick peas and lentils
- Other vegetables (e.g. parsnip/carrot)
### 3.17. Thinking about the PAST MONTH, please tick how often your baby has had the following drinks

<table>
<thead>
<tr>
<th>Yogurt and fromage frais</th>
<th></th>
<th></th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cooked Fruit</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Banana</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other fresh fruit</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bread or toast</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crackers or breadsticks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biscuits</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Drinks</th>
<th>Never</th>
<th>1-3 per month</th>
<th>Number of times per week</th>
<th>More than once a day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Baby juice</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pure fruit juice</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fruit drinks</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ribena, or high juice squash</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Squash, not including low calorie</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low calorie squash</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fizzy drinks, not including low calorie</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low calorie fizzy drinks</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tea</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

### 3.18. What does your baby have these drinks from?  

- **Bottle**  
- **Sippy/trainer cup**  
- **Cup**  
- **My baby doesn’t have these drinks**
YOUR BABY’S SLEEP

4.1 Where does your baby normally sleep?
- Infant crib in separate room
- Infant crib in parent’s room
- In parent’s bed
- Infant crib in room with sibling
- Other, specify

4.2 In what position does your child sleep most of the time?
- On his/her belly
- On his/her side
- On his/her back

4.3 How much time does your child spend in sleep during the NIGHT (between 7 in the evening and 7 in the morning)?

4.4 How much time does your child spend in sleep during the DAY (between 7 in the morning and 7 in the evening)?

4.5 Average number of night wakings per night:

4.6 How much time during the night does your child spend in wakefulness (from 10 in the evening to 6 in the morning)?

4.7 How long does it take to put your baby to sleep in the evening?

4.8 How does your baby fall asleep?
- While feeding
- Being rocked
- Being held
- In bed alone
- In bed near parent

4.9 When does your baby usually fall asleep for the night?

4.10 Do you consider your child’s sleep as a problem?
- A very serious problem
- A small problem
- Not a problem at all

4.11 How often does your baby snore?
- Never
- Sometimes (Less than once a week)
- Often (Once a week or more)
- Every Day
5 YOUR BABY’S BEHAVIOUR
We would like to ask about your baby’s behaviour.
As you read each description of the baby’s behaviour below, please indicate how often the baby did this during the LAST WEEK (the past seven days).

The “Does Not Apply” column is used when you did not see the baby in the situation described during the last week. For example, if the situation mentions the baby having to wait for food or liquids and there was no time during the last week when the baby had to wait, circle the “Does Not Apply” column.

“Does Not Apply” is different from “Never”. “Never” is used when you saw the baby in the situation but the baby never engaged in the behaviour listed during the last week. For example, if the baby did have to wait for food or liquids at least once but never cried loudly while waiting, circle the “Never” column.

<p>| 1 | When being dressed or undressed during the last week, how often did the baby squirm and/or try to roll away? | 1 | 2 | 3 | 4 | 5 | 6 | 7 | N/A |
| 2 | When tossed around playfully how often did the baby laugh? | 1 | 2 | 3 | 4 | 5 | 6 | 7 | N/A |
| 3 | When tired, how often did your baby show distress? | 1 | 2 | 3 | 4 | 5 | 6 | 7 | N/A |
| 4 | When introduced to an unfamiliar adult, how often did the baby cling to a parent? | 1 | 2 | 3 | 4 | 5 | 6 | 7 | N/A |
| 5 | How often during the last week did the baby enjoy being read to? | 1 | 2 | 3 | 4 | 5 | 6 | 7 | N/A |
| 6 | How often during the last week did the baby play with one toy or object for 5-10 minutes? | 1 | 2 | 3 | 4 | 5 | 6 | 7 | N/A |
| 7 | How often during the week did your baby move quickly toward new objects? | 1 | 2 | 3 | 4 | 5 | 6 | 7 | N/A |
| 8 | When put into the bath water, how often did the baby laugh? | 1 | 2 | 3 | 4 | 5 | 6 | 7 | N/A |
| 9 | When it was time for bed or a nap and your baby did not want to go, how often did s/he whimper or sob? | 1 | 2 | 3 | 4 | 5 | 6 | 7 | N/A |
| 10 | After sleeping, how often did the baby cry if someone doesn’t come within a few minutes? | 1 | 2 | 3 | 4 | 5 | 6 | 7 | N/A |
| 11 | In the last week, while being fed in your lap, how often did the baby seem eager to get away as soon as the feeding was over? | 1 | 2 | 3 | 4 | 5 | 6 | 7 | N/A |</p>
<table>
<thead>
<tr>
<th></th>
<th>Never</th>
<th>Very Rarely</th>
<th>Less Than Half the Time</th>
<th>About Half the Time</th>
<th>More Than Half the Time</th>
<th>Almost Always</th>
<th>Always</th>
<th>Does Not Apply</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>N/A</td>
</tr>
<tr>
<td>12</td>
<td>When singing or talking to your baby, how often did s/he soothe immediately?</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>13</td>
<td>When placed on his/her back, how often did the baby squirm and/or turn body?</td>
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</tr>
<tr>
<td>14</td>
<td>During a peekaboo game, how often did the baby laugh?</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>15</td>
<td>How often does the infant look up from playing when the telephone rings?</td>
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<td></td>
</tr>
<tr>
<td>16</td>
<td>How often did the baby seem angry (crying and fussing) when you left her/him in the crib?</td>
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<tr>
<td>17</td>
<td>How often during the last week did the baby startled at a sudden change in body position (e.g., when moved suddenly)?</td>
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<tr>
<td>18</td>
<td>How often during the last week did the baby enjoy hearing the sound of words, as in nursery rhymes?</td>
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<tr>
<td>19</td>
<td>How often during the last week did the baby look at pictures in books and/or magazines for 5 minutes or longer at a time?</td>
<td></td>
<td></td>
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<tr>
<td>20</td>
<td>When visiting a new place, how often did your baby get excited about exploring new surroundings?</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>21</td>
<td>How often during the last week did the baby smile or laugh when given a toy?</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>22</td>
<td>At the end of an exciting day, how often did your baby become tearful?</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>How often during the last week did the baby protest being placed in a confining place (infant seat, play pen, car seat, etc.)?</td>
<td></td>
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</tr>
<tr>
<td>24</td>
<td>When being held, in the last week, did your baby seem to enjoy him/herself?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>When showing the baby something to look at, how often did s/he soothe immediately?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>When hair was washed, how often did the baby vocalize?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>How often did your baby notice the sound of an airplane passing overhead?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>When introduced to an unfamiliar adult, how often did the baby refuse to go to the unfamiliar person?</td>
<td></td>
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</tr>
</tbody>
</table>

495
<table>
<thead>
<tr>
<th>Question</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>N/A</th>
</tr>
</thead>
<tbody>
<tr>
<td>29 When you were busy with another activity, and your baby was not able to get your attention, how often did s/he cry?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Never</td>
<td>Very Rarely</td>
<td>Less Than Half the Time</td>
<td>About Half the Time</td>
<td>More Than Half the Time</td>
<td>Almost Always</td>
<td>Always</td>
<td>Does Not Apply</td>
<td></td>
</tr>
<tr>
<td>30 How often during the last week did the baby enjoy gentle rhythmic activities, such as rocking or swaying?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>31 How often during the last week did the baby stare at a mobile, crib bumper or picture for 5 minutes or longer?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>32 When the baby wanted something, how often did s/he become upset when s/he could not get what s/he wanted?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>33 When in the presence of several unfamiliar adults, how often did the baby cling to a parent?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>34 When rocked or hugged, in the last week, did your baby seem to enjoy him/herself?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>35 When patting or gently rubbing some part of the baby’s body, how often did s/he soothe immediately?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>36 How often did your baby make talking sounds when riding in a car?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>37 When placed in an infant seat or car seat, how often did the baby squirm and turn body?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>38</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

|
### Appendix 6.2. Food items included in each food group of the FFQ at 6 months and number of missing values

<table>
<thead>
<tr>
<th>Food group</th>
<th>Food items included</th>
<th>Missing replies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allergenic foods (6 items)</td>
<td>Questions 3.6</td>
<td>14</td>
</tr>
<tr>
<td>Ready-made baby foods (12 items)</td>
<td>Question 3.16, 1st table</td>
<td>31</td>
</tr>
<tr>
<td>Starchy foods (8 items)</td>
<td>Questions 3.16, part of 2nd table (Weetabix, other cereals, potatoes, rice, pasta, bread/toast, crackers/breadsticks, biscuits)</td>
<td>24</td>
</tr>
<tr>
<td>Vegetables (2 items)</td>
<td>Questions 3.16, part of 2nd table (beans/pulses, other vegetables)</td>
<td>3</td>
</tr>
<tr>
<td>Fruits (3 items)</td>
<td>Questions 3.16, part of 2nd table (cooked fruit, banana, other fresh fruit)</td>
<td>4</td>
</tr>
<tr>
<td>Yogurt and fromage frais (1 item)</td>
<td>Questions 3.16, part of 2nd table</td>
<td>4</td>
</tr>
<tr>
<td>Meat/Fish (2 items)</td>
<td>Questions 3.16, part of 2nd table</td>
<td>8</td>
</tr>
</tbody>
</table>