UNIVERSITY OF SOUTHAMPTON

FACULTY OF MEDICINE

Academic Unit of Clinical and Experimental Sciences

Infant Feeding Practices in the First Year of Life and their Relationship with the Development of Allergic Disease by the Age of Two Years

by

Kathryn Elizabeth Courtiour Grimshaw

Thesis for the degree of Doctor of Philosophy

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ABSTRACT

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Doctor of Philosophy

INFANT FEEDING PRACTICES IN THE FIRST YEAR OF LIFE AND THEIR RELATIONSHIP WITH THE DEVELOPMENT OF ALLERGIC DISEASE BY THE AGE OF TWO YEARS.

by Kathryn Elizabeth Courtiour Grimshaw

Food allergy has significant individual, household and societal costs with the increasing prevalence of allergic disease becoming a public health concern, particularly in Westernised, urbanised countries. Consequently there is great interest in the aetiology of food allergy in general, and the primary prevention of food allergy in particular.

This study set out to determine whether specific aspects of infant feeding in the first year of life were associated with the development of food allergy by two years of age. Children who developed food allergy, defined using double blind placebo controlled food challenges, were compared with two age matched controls. All infants were part of a larger cohort study (PIFA Study). The mothers of all study infants (n=123) prospectively kept weekly diaries of all foods consumed and breast milk and infant formula consumption. Diet data was initially assessed using a purpose designed, unique data entry package which then allowed the resultant data to be analysed using a number of different methodologies including descriptive statistics, survival analysis, repeated measures ANOVA, Principal Component Analysis and logistic regression analysis.

Three main associations between infant feeding in the first year and allergy development were found. These were: an 'healthy' infant diet pattern which followed infant feeding recommendations was associated with reduced allergy risk; introduction of solids before 17 weeks of age was associated with increased allergy risk; and concurrent breastfeeding and introduction of cows' milk protein was associated with reduced allergy risk.

Consequently, the data from this study, particularly those regarding the protective effect of a 'healthy' weaning diet pattern, have provided some unique findings that can support current infant feeding and allergy prevention recommendations and can inform further research in the field.

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DECLARATION OF AUTHORSHIP

I, Kathryn Elizabeth Courtiour Grimshaw,

declare that the thesis entitled

Infant Feeding Practices in the First Year of Life and their Relationship with the Development of Allergic Disease by the Age of Two Years

and the work presented in the thesis are both my own, and have been generated by me as the result of my own original research. I confirm that:

- this work was done wholly or mainly while in candidature for a research degree at this University;
- where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
- where I have consulted the published work of others, this is always clearly attributed;
- where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
- I have acknowledged all main sources of help;
- where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
- none of this work has been published before submission,

Signed:

Date: 2nd May 2012

Acknowledgements

I would like to acknowledge the work of the PIFA Study team and Steering group as without the PIFA Study I would have no data!

I would also like to thank the Winchester and Eastleigh midwives, and the parents and infants who participated in the PIFA study. Without them there would be no infants enrolled in the study and no diet diaries and I would have no data!

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Even with data, I would not have been able to produce this thesis without the help, support and guidance of my supervisor Barrie Margetts. Thank you.

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Last, but no means least, thanks to Charley just for being you and surrounding me with your cheerfulness, and thanks to Grim who is probably the best proof reader 'in the world'!!!

List of Definitions and Abbreviations

Definitions

Cessation of breastfeeding – breastfeeding is completely stopped.

Food Hypersensitivity – a hypersensitivity reaction to a food or food ingredient where immune mechanisms are involved. It is either IgE mediated or non IgE mediated

Initiation of breastfeeding – breastfeeding was started with the infant receiving at least one breastfeed.

Introduction of infant formula – where the infant first receives a modified cow's milk formula. This can be whey or casein based. Also includes 'follow-on' modified cow's milk formulae.

Introduction of solids – where the infants first receives solid/semi-solid foods.

Overlap feeding of breast milk and solids – infants receives some breast milk and some solids/semisolids in the same 24 hour period. (Proportions need not be specified).

Transition foods – solid or semi-solid foods that are specifically formulated for infants (either nutritionally or physically). These can be commercial or home-prepared.

Abbreviations

AD	Atopic Dermatitis
ANOVA	Analysis of Variance
APC	Antigen Presenting Cell
CI	Confidence Interval
COLAP	Colonoscopic Allergen Provocation
COT	Committee on Toxicity of Chemicals in Food
DBPCFC	Double Blind Placebo Controlled Food Challenge

DLW	Doubly Labelled Water
DoH	Department of Health
EAACI	European Academy of Allergy and Clinical Immunology
EAR	Estimated Average Requirements
EFSA	European Food Safety Authority
FcεRI	High Affinity Receptor
FFQ	Food Frequency Questionnaire
FSA	Food Standards Agency
GALT	Gut Associated Lymphoid Tissue
GINI	German Infant Nutritional Intervention Study
IFN-γ	Interferon γ
IFS	Infant Feeding Study
IgA	Immunoglobulin A
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL-2	Interleukin-2
IL-4	Interlukin-4
IL-10	Interleukin-10
IL-12	Interleukin-12
IQR	Interquartile Range
ISAAC	International Study of Asthma and Allergy in Childhood
LCPufas	Long Chain Poly Unsaturated Fatty Acids
LT	Leukotriene
MHC	Major Histocompatibility Complex
NAFH	Non-allergic Food Hypersensitivities
NCT	National Childbirth Trust
NSAIDs	Non-steroidal Anti-inflammatory Drugs
PCA	Principal Component Analysis
PG	Prostaglandin (PG)
PIFA	Prevalence of Infant Food Allergy
Pufas	Poly Unsaturated Fatty Acids

RHCH	Royal Hampshire County Hospital
RM-ANOVA	Repeated Measures Analysis of Variance
RNI	Reference Nutrient Intake
SACN	Scientific Advisory Committee on Nutrition
sCD14	Soluble CD14
SCORAD	Scoring Atopic Dermatitis
SD	Standard deviation
sIgA	Soluble Immunoglobulin A
SpIgE	Serum Specific IgE
SPT	Skin Prick Testing
TCR	T Cell Receptor
TGF- β	Transforming Growth Factor- β
Th	T Helper
Treg	Regulatory T Cell
WAO	World Allergy Organisation
WTCRF	Wellcome Trust Clinical Research Facility
WHO	World Health Organisation

1. Introduction

Food allergy is a food hypersensitivity reaction where immune mechanisms are involved (1). The prevalence of food allergy is widely believed to be increasing (2), (3) but since the term has differing meanings to different clinical specialisms and there are also varying levels of diagnosis from patient perceived to diagnosis by double blind placebo controlled food challenge (4), an accurate picture of prevalence is hard to ascertain. However, it is widely accepted that it is most commonly acquired during the first year of life with peak incidence of 6-8% occurring at one year of age. The prevalence then falls until late childhood where it plateaus at 1-2% and remains at that level throughout adulthood (5).

Although many children grow out of their reactions to food, it is evident that allergic disorders change and progress from eczema and food allergy to asthma, rhinitis and inhalant allergy. This experience is referred to as the 'allergic march' and is a commonly demonstrated phenomenon (6). Prevention of the first stage of the allergic march where the body reacts to a food protein after being exposed to it for the first time seems the obvious course of action when trying to reduce the prevalence of allergic diseases. The infant's immune system first comes into contact with food allergens as a foetus (7), continues being exposed after birth via breast milk (8), and then again when solids are introduced into the diet. It is unclear which, if not all of these exposure time points are important in initiating the allergic march and is why prevention strategies tend to focus on pregnancy and the first year of life (9).

The question of how best to feed a child to reduce the risk of allergy development is a matter of great debate. This study aimed to determine whether infant feeding in the first year of life was associated with the development of food allergy by two years of age. Its focus is on infant exposure to food allergens via oral ingestion after birth. However, since exposure via the mother during pregnancy may have a role in the development of food allergy, this was also considered but to a lesser extent.

The study design was a case control within a cohort study hence dietary intake data was collected prospectively from birth in the same manner for all infants whether they subsequently developed symptoms of food allergy or not (which limits recall bias). Information on when symptoms first started was also collected prospectively in order to control for differences in feeding patterns resultant from early symptoms of food allergy.

1.2 Hypothesis

1.2.1 Overall hypothesis

Children who are diagnosed with a food allergy by the age of 2 years will have been fed differently in the first year of life compared to children who have not been diagnosed as food allergic by the age of 2 years (control children).

1.2.2 Specific Hypotheses:

1a. Children who develop food allergy by two years of age will have a 5% lower rate for the initiation of breast feeding than children who do not develop a food allergy.

1b. Mean breastfeeding duration for children who develop food allergy by two years of age will be 4 weeks less than for children who do not develop a food allergy.

1c. Mean exclusive breastfeeding duration for children who develop food allergy by two years will be 4 weeks less than for children who do not develop a food allergy.

1d. Mean duration of concurrent breastfeeding with any cows milk protein for children who develop food allergy by two years will be 4 weeks less than for children who do not develop a food allergy.

2. Children who develop food allergy by two years of age will have solid foods introduced into their diet a mean of 4 weeks earlier than children who do not develop a food allergy.

3. Children who develop food allergy by two years of age will have a 0.5 standard deviation difference between the mean intake of certain macro and mico-nutrients. Nutrients included in the analysis are energy, protein, fat, iron, zinc, selenium, vitamin A, vitamin C and vitamin E than children who do not develop a food allergy.

4. Children who develop food allergy by two years of age will have a 0.5 standard deviation difference in their PCA pattern score than children who do not develop a food allergy.

[Details of the rationale behind the detail behind these hypotheses are given in Section 3.2].

1.3 Aim

To determine whether specific aspects of infant feeding in the first year of life are associated with the development of food allergy by two years of age.

1.4 Objectives

1. Collection of prospective food diary data for infants recruited onto the PIFA study.

2. Design of a data analysis technique to capture dietary pattern data from prospective food diaries.

3. Determination of mean daily nutrient intake data from quantitative prospective food diary data for infants diagnosed with food allergy and their two-age matched controls.

4. Use of prospective food diary data to determine the nature of the weaning diet for children diagnosed with food allergy by the age of 2 years and their two-age matched controls.

5. Use of prospective food diary data to identify dietary patterns in children diagnosed with food allergy by the age of 2 years and their two-age matched controls.

1.5 Layout of Thesis

Chapter 2 reviews the topic of food allergy. It will describe the basic immunology behind food allergic reactions and the concept of oral tolerance. Since the scope of this thesis is to focus on the nutritional aspects of the relationship between infant feeding and food allergy development, the mechanistic details of the immune response and oral tolerance will be not be described in great detail in this thesis although an overview will be given. Prevalence of food allergy will then be discussed followed by a detailed section on allergy prevention strategies. Allergy prevention strategies are concerned with early life factors, particularly nutrition related factors such as maternal

nutrition during pregnancy, duration and exclusivity of breast feeding, incidence and duration of formula feeding, and the timing and nature of the introduction of solids.

Since maternal nutrition is thought to have a role in the development of food allergy (10) research into its role in allergy development (or prevention) will be discussed in section 2.7.1. However, as the role of maternal nutrition is not directly related to any of the thesis objectives, the topic is not being fully researched but study findings related to maternal nutrition will still be presented in section 5.1 and will be discussed in section 6.4.

The question of how best to feed a child to reduce the risk of allergy development is a matter of great debate. Whilst it is established that breast feeding is generally the best way to feed an infant, there is no consensus as to how long infants should be breastfed to reduce allergy risk (11) nor whether it is beneficial to continue to breastfeed when solids are being introduced as many immunologically active compounds present in breast milk have been shown to modify immune system development (8). This will be looked at in greater detail in section 2.7.2.

Another area of debate is when to introduce solids and whether the introduction of allergenic foods should be delayed as an allergy prevention measure. Most intervention studies have included delaying the introduction of solids as part of the experimental protocol either as part of the intervention in association with other modifications to the diet and environment (12;13) or as advice for all study groups (14). Thus it is not possible to determine in isolation the influence of solid introduction on the later development of adverse reactions to foods from these studies. Despite this absence of evidence, delaying the introduction of solids into an infant's diet has been widely advocated as an allergy prevention measure (15) with the additional recommendation that the introduction of allergenic foods should be further delayed. The literature relating to current thinking regarding when solids should be introduced as an allergy prevention measure will be dealt with in section 2.7.4.2.

Since food allergy diagnosis has different methodologies, it is important for the study's food allergy outcome measures to be robust, accurate and reproducible. Furthermore, it is essential that the dietary assessment tools used result in the collection of accurate dietary intake data from which further analyses can be carried out. Collection of accurate and fit-for-purpose dietary intake data is notoriously difficult (16) and careful consideration during planning was necessary to ensure the methodology delivered appropriate and analytical data to support the four study objectives. The issues surrounding methodological selection and rationales and validity and reliability of the data collected are detailed in chapter 3.

The study methodology will be described in chapter 4. Chapter 5 will detail the results where the results pertaining to each Hypothesis will be dealt with separately (sections 5.3 to 5.6). Finally the results of the study are discussed in light of the relevant literature in chapter 6 along with a discussion of limitations of the study. Conclusions about the study and its contribution to the existing field of knowledge and recommendations for further work in this area are made in chapter 7.

2. LITERATURE REVIEW – Food Allergy

2.1. Search strategies

Firstly, general text books on food allergy, paediatric food allergy, infant and maternal nutrition were accessed. The relevant chapters were read (e.g. mechanisms of food allergy, oral tolerance in food allergy, prevention strategies in food allergy) and details of cited articles noted. These articles were then read to piece together an overview of the topic. An electronic search of renowned databases (PUBMED, MEDLINE, EMBASE, COCHRANE,) was then carried out. Terms inputted were 'food allergy', 'oral tolerance', 'allergy prevention', 'weaning', 'Complementary feeding', 'breast feeding', 'formula feeding', 'infant feeding', 'prevalence', and 'maternal nutrition' The terms were used singly or with others to refine the searches further. Where the title of the paper seemed relevant the abstract was read, if after this the paper continued to seem to be relevant the whole paper was read. Relevant articles referred to from original papers read where also obtained and read.

Details of the main papers reviewed (including levels of evidence) are detailed in Appendix 1

2.2 Definition of Food Allergy and food allergy terms

2.2.1 Definition of food allergy

The term 'food allergy' is widely used and is often inaccurately applied; therefore it is important to define the disease phenotype that will be applied to this work. The World Allergy Organisation published a revised nomenclature for adverse reactions to food in 2003 (1) which is detailed in Figure 2.1. It defines Food Allergy as a hypersensitivity reaction to a food or food ingredient where immune mechanisms are involved. It is either IgE mediated or non IgE mediated (Figure 2.1) although in some conditions both mechanisms are thought to act (17). The revised nomenclature goes on to specify that food hypersensitivity reactions that do not involve the immune system are to be termed non-allergic food hypersensitivities (NAFH) and that reactions to foods that are not reproducible by double blind placebo controlled food challenge are considered food aversions.

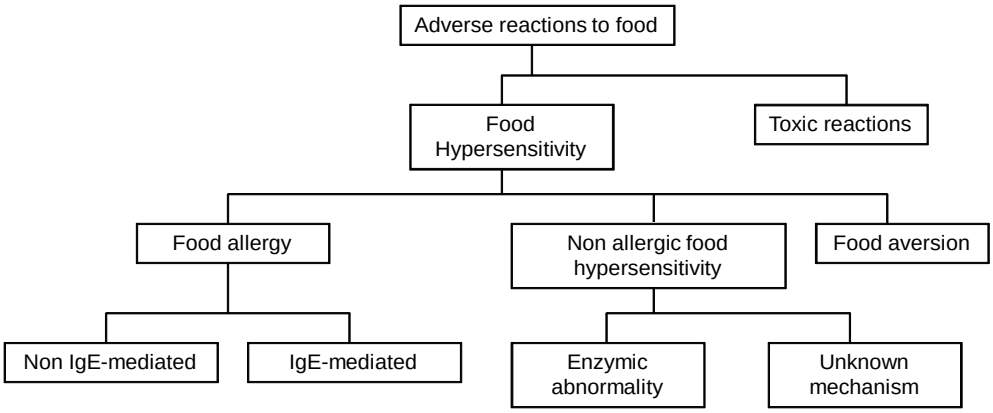

Figure 2.1 Flowchart showing nomenclature used in food hypersensitivity reactions. *Diagrammatic representation of nomenclature to be used in food hypersensitivity reactions where reactions not reproducible under double blind conditions are termed food aversions. Reactions with an immunological mechanism are termed food allergy. Reactions without an immunological mechanism are non-allergic food hypersensitivity (NAFH) reactions. (1)*

Although this classification system is helpful, it is likely that some reactions that are not currently thought to be due to IgE may be shown to be so as diagnostic practices and immunological knowledge improve. Gastro-intestinal reactions fall into this category as the inflammatory factors (e.g. IgE and eosinophils) may only be found in the gut and cannot always be identified in either serum or skin. The only way to demonstrate their presence is to carry out intestinal biopsy or colonoscopic allergen provocation test (COLAP test) (18), but both of these procedures are currently considered too invasive for routine diagnostic purposes so in practice the term food allergy is often all that can be applied. The WAO definition of food allergy (1) has been supported by the new United States of America guidelines which states Food Allergy as 'an adverse health effect arising from a specific immune response that occurs reproducibly on exposure to a specific food' (19).

Food allergic reactions can occur immediately after a food is eaten or they can take up to 48 hours to manifest and they may be allergen dose dependant. Any reactions that occur beyond this timeframe are unlikely to be due to a food allergy but are more likely to be due to a non-allergic food hypersensitivity reaction (NAFH) which are reactions that are reproducible under double blind food challenge conditions but do not have an immunological mechanism (20). Such reactions may have a known mechanism such as enzyme deficiency but for many the mechanisms are unknown.

The onset of NAFH may go unnoticed as initial symptoms may be mild but they usually increase in severity and often in number with developing intolerance to more and more foods over time. There may be no preceding event prior to onset but in some cases there is an obvious incident such as a severe bout of food poisoning. Most NAFH (except enzyme deficiencies and adverse reactions to food additives) are not found in infants or young children so these type of food hypersensitivity reactions will not be further considered in this thesis.

2.2.2 Definition of food allergy terms

There are many terms used in the food allergy field. It is useful to clarify the use of these terms in the context of this thesis to aid comprehension of the later sections.

Allergen – any substance that can cause an allergy
Allergic disease – clinical conditions caused by allergy
Allergic rhinitis – inflammation of the mucous membrane lining the nose caused by an allergy
Antigen – any substance that can cause an immune response in the human body
Asthma – respiratory disorder characterized by wheezing, usually of allergic origin
Atopy – a hereditary tendency to be hypersensitive to certain allergens
Atopic dermatitis – a severe condition of inflamed skin (eczema) characterised by atopy
Eczema – generic term for inflammatory conditions of the skin
Food Allergy – a hypersensitivity reaction to a food or food ingredient where immune mechanisms are involved.
Hydrolysed infant formula – infant formula that has been treated so the protein has been split into shorter length peptides
IgE – the immunoglobulin involved mechanistically in IgE mediated food allergies
IgE mediated food allergy – a hypersensitivity reaction to a food or food ingredient where IgE can be demonstrated to have a role (either by skin prick test or serum specific IgE measurement).
Non-IgE food allergy – a hypersensitivity reaction to a food or food ingredient where immune mechanisms are involved but IgE cannot be shown to be acting.
Skin prick test – A non-invasive diagnostic procedure used to determine the presence of IgE. Provides a result within 15 minutes but is not 100% positively or negatively predictable
Serum Specific IgE – A blood measurement of levels of IgE which is specific to a certain antigen (as opposed to total serum IgE). Levels above 0.35kU/l, are taken as confirming allergy to that antigen
Wheeze – breathing with a husky or whistling sound

2.3 Definition of Infant feeding terms

Terms surrounding infant feeding are frequently used but their meanings differ widely. The term 'weaning' demonstrates this with differing definitions including: 'permanent deprivation of breast milk' (21); and 'to accustom to food other than mothers milk' (22). It is apparent when reading the medical literature there has been no consensus as to the meaning of terms used to describe infant feeding such as, in particular, 'weaning' and 'complementary feeding'. In an effort to change the impression that solid food introduction should displace breast milk intake, the World Health Organization (WHO) have moved away from using the term 'weaning' to describe the introduction of solids and semi-solids into the infants diet and instead advocate the use of the term 'complementary feeding' (23). Up to this point, when authors were describing the process of expanding a child's diet from receiving breast and/or formula milk to one that contains solid/semi-solid food they would use the term 'weaning' (24;25). Whilst the medical literature has to some extent embraced using the term 'complementary feeding' (26) (27) (28) there are still research papers using the term 'weaning' to describe the introduction of solids into an infant's diet (29;30) which is continuing the confusion around the correct meaning of these terms.

Demonstrating this confusion, WHO define 'weaning' as the process of stopping breastfeeding with the term 'weaned' referring to when breastfeeding stops (23) and this is the meaning conferred by Rogers, Emmett and Golding (31) and Lowe, Hosking, and Bennett et al (32) whereas other researchers use the term to describe introduction of solids (29;30;33). Similarly, 'complementary feeding' is defined by WHO as when nutritive solids, semi-solids or liquids are given in addition to breast milk (23). However researchers using this term generally use it to mean when solids or semi-solids (i.e. not a liquid) are introduced into an infant's diet regardless of whether the infant is receiving breast milk (26-28).

Some papers avoid the terms 'weaning' and 'complementary feeding' altogether and instead use phrases which are not so open to misinterpretation such as 'age at solid introduction', 'cessation of breastfeeding' and 'introduction of infant formula' (34;35). This will be the case in this thesis with terms and meanings used as follows:

'Initiation of breastfeeding' – breastfeeding was started with the infant receiving at least one breastfeed.
'Cessation of breastfeeding' – breastfeeding is completely stopped
'Overlap feeding of breast milk and solids' – infants receives some breast milk and some solids/semisolids in the same 24 hour period. (Proportions need not be specified)

'Introduction of infant formula' – where the infant first receives a modified cow's milk formula. This can be whey or casein based. Also includes 'follow-on' modified cow's milk formulae.

'Introduction of solids' – where the infants first receives solid/semi-solid foods.

'Transition foods' – solid or semi-solid foods that are specifically formulated for infants (either nutritionally or physically). These can be commercial or home-prepared.

2.4 Development of the immune response and mechanisms of food allergy

In normal circumstances food is ingested by an individual and it does not cause an allergic reaction. This lack of a reaction is not a passive process but is an active non-response of the immune system to the antigen and is termed oral tolerance. Oral tolerance is considered to be both an adaptive and an innate immune, but active non-response.

For the immune system to respond (as in allergy) or actively not respond (as in oral tolerance) to a food the immune system needs to be educated about its surroundings and this occurs via primary and secondary sensitisation.

2.4.1 Primary and secondary sensitisation (primary and secondary immune response)

Together with the skin, mucosal surfaces of the gastrointestinal and respiratory tracts provide the principal interface between the immune system and the outside environment, so highly efficient antigen surveillance mechanisms are required to maintain immunological tolerance. The key cell involved is the dendritic cell which is a highly specialised antigen presenting cell (APC). After antigen uptake, the APC internalises the antigen, degrades it into short peptides of 15 to 20 amino acids, and presents these peptides as part of the major histocompatibility complex (MHC) on its cell surface. The APC then moves to the lymph node where allergen presentation to the naïve T helper (Th) cells causes activation, cytokine production and generation of memory Th cells which are released into the circulation.

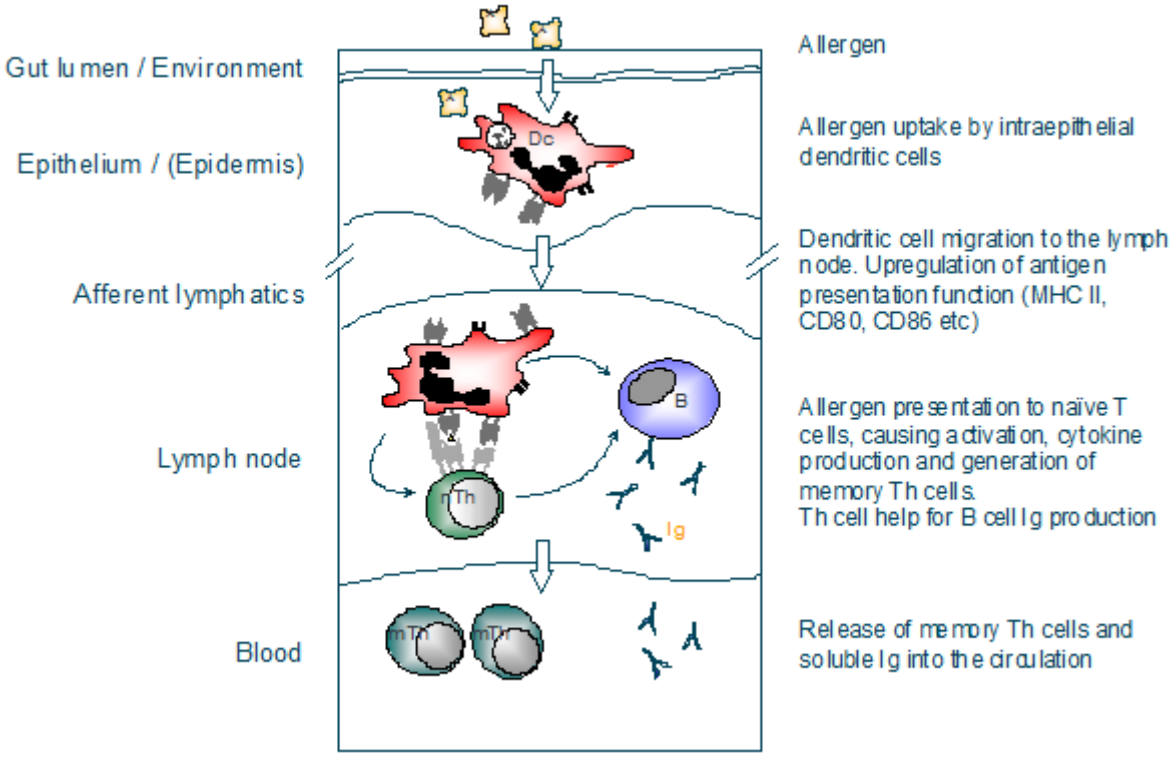

Figure 2.2 Overview of steps involved in Primary Sensitisation (primary immune response). *Figure shows allergen uptake by intraepithelial dendritic cell and then migration of dendritic cell to lymph node where it presents allergen to naïve T cell causing activation. (Figure kindly supplied by Dr J A Holloway, Course director MSc Allergy, University of Southampton).*

Th cell activation is not just a matter of cell-cell contact between the Th cell and the APC (by way of MHC and the T cell receptor (TCR)), there needs to be co-stimulation which increases the strength of the activation signal and this occurs due to ligation between CD80/CD86 on the APC and CD28 on the Th cell. The activation is also affected by the cytokine environment which mediates effects by specific cytokine actions. After activation, the Th cells 'help' the immature B cells in the lymph node to make antibodies. The type of antibody they make is dependent on the cytokine environment. These antibodies are then released into the circulation.

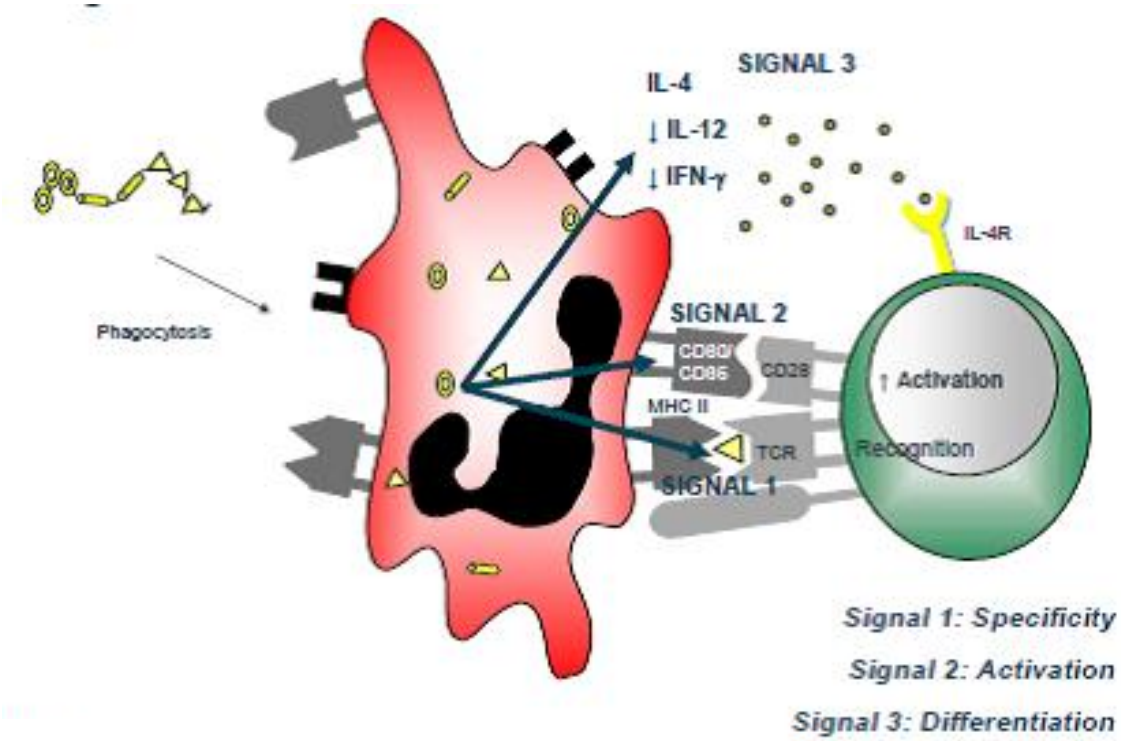

Figure 2.3 Diagrammatic representation of Th cell activation. *Figure represents interaction between APC and Th cell including link between MHC class II and TCR, ligation between CD80/CD86 on the APC and CD28 and the cytokine environment. (Figure kindly supplied by Dr J A Holloway, Course director MSc Allergy, University of Southampton).*

When memory B or T cells encounter antigen for a second time the resultant response is quicker, and stronger. This is secondary sensitisation.

2.4.2 Mechanisms of food allergy

Whether an allergic reaction involves IgE or not is used to differentiate between the types of food allergic reactions (Figure 1). Sensitisation occurs in the same manner for both types. In IgE mediated allergy the Th cell interacts with the B cell causing IgE production. In non IgE mediated allergy the Th cell interacts directly with inflammatory cells such as eosinophils leading to many of the observed symptoms of non-IgE mediated disease.

2.4.2.1 IgE Mediated Food Allergy

IgE mediated responses to food are bi-phasic with the early phase response occurring within an hour of encounter with the allergen (often within minutes) and the late phase response occurring 4-8 hours later. IgE is an immunoglobulin normally present at very low levels in the plasma of non-allergic individuals, but levels are often raised in patients suffering from allergic conditions such as asthma, atopic dermatitis and anaphylaxis. IgE has a very short life in plasma (T ½ less than 1 day) but can

remain coupled to receptors on tissue resident mast cells for weeks or months. IgE antibodies exert their biological function via the high affinity receptor FcεRI on mast cells, dendritic cells and basophils and the low affinity receptor CD23 on B cells, macrophages, monocytes, lymphocytes, eosinophils and platelets. When food allergens penetrate mucosal barriers and reach food specific IgE antibodies bound to mast cells or basophils via the FcεRI receptor, they cross link between two IgE molecules which causes signaling and degranulation of the cells which results in the release of pre-formed and newly synthesized vasoactive and chemotactic mediators. These induce vasodilation, vascular leakage, smooth muscle contraction and mucus secretion which result in symptoms of the early phase response.

The activated mast cells also release a variety of cytokines and chemokines, which can induce the IgE-mediated late phase response by attracting inflammatory cells to the site of reaction. During the initial 4-8 hours, neutrophils and later eosinophils migrate to the site of response. These infiltrating cells are activated and release a variety of mediators including oxygen radicals, platelet activating factor, peroxidase, eosinophil major basic protein and eosinophil cationic protein, causing tissue damage aggravating the inflammation. In the subsequent 24 to 48 hours, lymphocytes and monocytes infiltrate the area and establish a more chronic inflammatory environment.

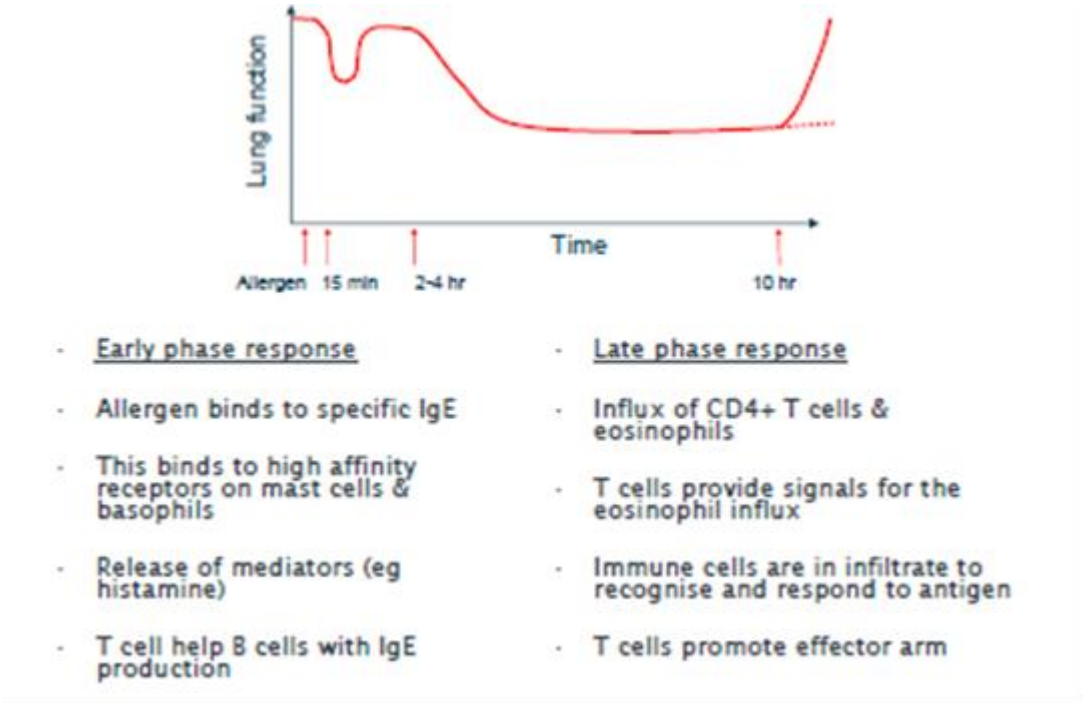

Figure 2.4 Time-line showing early and late phase response. *Figure shows the kinetics of the immune response is biphasic with the late phase response taking place 2-4 hours after ingestion. (Figure kindly supplied by Dr J A Holloway, Course director MSc Allergy, University of Southampton).*

2.4.2.1.1 Clinical manifestation of IgE mediated food allergy

Symptoms generally appear within minutes after a very small amount of food has been consumed. Immediate symptoms may affect the gut, the skin and/or the lungs depending on which tissues the mast cells are present. Symptoms include vomiting, abdominal pain, urticaria, angio-oedema (swelling of the face/throat), erythema, pruritis, wheeze and cough.

Symptoms of upper airway obstruction (laryngeal oedema), lower airway obstruction (broncho-constriction), hypotension, cardiac arrhythmias and even heart failure constitute the most severe and life-threatening reactions. This is known as anaphylactic shock and first symptoms may include sneezing and a tingling sensation on the lips, tongue and throat followed by pallor and feeling unwell, fearful, warm and lightheaded. Generalised erythema, urticaria and angio-oedema are common features but do not always occur. Severe symptoms may recur up to 6 hours after apparent resolution due to the late phase response. The presentation of anaphylaxis is varied but the major causes of death are obstruction to the upper airway or shock, hypotension and cardiac arrhythmia (36).

The results of DBPCFC from several studies in the USA have shown that the most common foods to provoke immediate reactions are peanuts, tree nuts, milk, egg, soy and wheat, fish and shellfish (37). Seeds (e.g. sesame) have also been implicated.

2.4.2.2 Non-IgE mediated Food Allergy

Reactions to food where IgE isn't present are poorly defined both clinically and scientifically. Reactions are believed to be Th cell mediated where the Th cell interacts directly with inflammatory cells such as eosinophils causing infiltration of these cells into tissues. The reason the T cell reacts in this manner is due to the cytokines involved namely interferon γ (IFN-γ) and Interleukin- 12 (IL-12). Another possible mechanism of non-IgE mediated disease is when the B cell class-switches to produce antigen specific Immunoglobulin G (IgG) which can bind with the specific antibody causing neutrophil infiltration and release of tissue damaging lysosymal granules.

2.4.2.2.1 Clinical manifestation of non-IgE mediated disorders

These reactions are variable, they may develop slowly after hours or days and only after a threshold dose of allergen has been consumed (38). As with immediate symptoms, adverse reactions can affect the skin, gut or lungs. Symptoms can include chronic diarrhoea, abdominal pain, failure to thrive, enteropathy, allergic eosinophilic oesophagitis and gastritis, gastroenteritis, allergic colitis, infantile colic, constipation, eczema and asthma.

Foods most commonly causing these reactions are milk, egg, soya and wheat. Enterocolitis reactions have been shown to be due to grains, fish and poultry ingestion (39).

2.5 Development of Oral tolerance in Food Allergy

It is commonly accepted that initial sensitization to a food allergen happens via the gut or through sub-cutaneous exposure (40). In the vast majority of cases, food antigens are ingested without causing adverse reactions of any kind. As mentioned earlier, this lack of a reaction is not a passive situation but is an active non-response of the immune system to the antigen and is termed oral tolerance. Current knowledge suggests there are multiple pathways to achieving oral tolerance which can be divided into two types, central tolerance where clones of cells that have receptors for self-antigens are deleted during development in the thymus and peripheral tolerance where circulating Th cells are involved.

2.5.1 Pathways of Peripheral Tolerance

2.5.1.1 Anergy

This is a state of un-responsiveness where the TCR is stimulated but the Th cell either does not receive any co-stimulation or its CTLA-4 is up-regulated and binds with CD80/86. Lacking the appropriate activation, the T cell is incapable of responding to the antigen and mounting an immune response. Additionally, repeated stimulation of these T cells induces Interlukin-2 (IL-2) release, and increases T cell sensitivity to apoptosis (see *2.5.1.3 deletion*).

2.5.1.2 Suppression

Th cells can also provide a regulatory function in the form of regulatory T cells (Treg) which are a subset of Th cells. There are 3 types of Treg cells; natural which are made by the thymus as functionally mature cells and which are non-proliferative, inducible Treg cells which are antigen specific and Natural Killer T cells which have a major regulatory role in autoimmunity. All Treg cells suppress the activation of other lymphocytes and by promoting a cytokine environment rich in Interleukin-10 (IL-10), Transforming Growth Factor- β (TGF- β) and low in IL-2 and Interleukin-4 (IL-4).

2.5.1.3 Deletion

Repeated stimulation of mature Th cells induces IL-2 release and up-regulates the *Fas* ligand, increasing sensitivity to apoptosis (deletion).

2.5.1.4 Ignorance

As the name suggests, this is where the immune system does not respond to an antigen as it has not previously encountered it.

2.5.2 Gut mucosa in oral tolerance

In normal circumstances food antigens are digested and do not enter directly into the cells of the intestinal wall due to the physical barrier of mucus and the action of Immunoglobulin M (IgM) and Immunoglobulin A (IgA) which provide an immunological barrier along the gut lumen. Food antigens can however pass through the intestinal wall after being taken up by dendritic cells which have processes ('fingers') protruding into the lumen of the gut, or via the M cells of the Peyers patch. These M cells sample the contents of the intestinal mileau and transcytose proteins they have sampled passing on resultant peptides (termed antigen) to dendritic cells and macrophages found in the sub-epithelial M cell pocket. Peptides taken up in this manner are brought into the Peyers patch where they elicit an IgA response via IgA committed B cells which enter the lymphatic system where they can mediate a wider gut response to the offending antigen. If the antigen bypasses processing by the M

cells, then professional antigen presenting cells will encounter it and present it to T cells of the lamina propria along with major histocompatability complex class 2 as described in section 2.4.1.

2.5.3 Breast milk in oral tolerance

Breast milk is thought to play an important role in the development of oral tolerance as there are a number of components in breast milk that are important in protecting the infant from microbial/antigen attack (41). Some of these factors initiate the innate immune response but some are closely involved in the development of the infant's adaptive immune system. Immunological factors in breast milk consist of maternal immunoglobulins, oligosaccharides, cytokines, glycoproteins, long chain poly unsaturated fatty acids (LCPufas), lysozyme, nucleotides and complement. Many of these are thought to have a role in the development of oral tolerance, in particular TGF- β which has an positive association with infant wheeze (42) and atopic eczema (43), IL-12 which promotes Th-1 cytokine mileau (44) and soluble CD14 (sCD-14) whose levels in breast milk have been shown to be negatively associated with eczema at 6 months of age (45).

Additionally, Secretory IgA (sIgA) in breast milk is thought to shield the breast-fed infant's gut-associated lymphoid tissue (GALT) from dietary allergens, thereby reducing local immunostimulation and contributing to the establishment of oral tolerance (8). Findings to support this theory are that breastfeeding has been shown to significantly protect against the development of coeliac disease in children, an effect that is unrelated to the time of solid food introduction (46) and that the infants of mothers with relatively low levels of milk IgA antibodies to bovine proteins are more likely to develop cow's milk allergy (47;48). However, other researchers have not found any association between breast milk cytokines and allergy outcome (49;50). This has been explained by the fact that levels of these cytokines differ vastly between breast milk samples and when levels of cytokines in the milk is taken into account then a relationship is seen (51).

2.6 Prevalence of Food allergy

Around 1-2% of the European adult population are estimated to suffer from food allergy (specifically the IgE-mediated form)(52). Since reports of food allergies have only become apparent in the last 20 years (53-56), prevalence is perceived to be increasing. They are a cause of particular concern for young children where the prevalence of food allergy (often life-threatening) is estimated to be 5-8% for toddlers compared to 1-2% for adults (52;57-60). A meta-analysis was carried out which found the prevalence of food allergy in childhood to be around 3% with the important

allergens in infancy being milk, egg and peanuts (4). The meta-analysis used a definition that included food associated symptoms and sensitisation as defined by a positive skin prick test or serum specific IgE which may lead to an under-estimation of the number of infants with food allergy as it does not consider those infants with Non-IgE mediated food allergy.

However the meta-analysis demonstrated that whatever definition is used, prevalence rates differed between countries, an observation previously identified by a number of authors (61-64). Whilst differences may be due to different study design, there is a belief that food allergy rates do differ between countries (19) and that these differences may be due to both constitutional and environmental factors (65). It is unlikely that any one of these factors on their own is solely responsible for allergy development, particularly since studies looking at specific allergy risk factors such as probiotic use (15)), polyunsaturated fatty acid intake (66), pet ownership (67), bacterial exposure (68), environmental pollutants (69), and topical cream use (40) have not delivered conclusive results. Hypotheses considering potential interactions between such 'risk' factors and also 'protective' (tolerance inducing) factors may be more effective at improving knowledge in this area (70).

2.7 Prevention of Food Allergy

Allergy prevention may be sub-divided into three stages, primary, secondary and tertiary Figure 2.5 (71). Thus primary prevention occurs before primary sensitisation of the immune system. In general, such prevention strategies are likely to focus on pregnancy and the first year of life. Secondary prevention involves strategies directed at preventing disease symptoms resulting from primary sensitisation of the immune system and these interventions are likely to be most effective when employed in the first years of life. Tertiary prevention involves the use of strategies put in place after the development of the first signs of allergic disease in an attempt to stop the development of new disease symptoms and to control existing symptoms. At present this mostly involves the use of pharmaceutical products. Prevention of the first stage of the allergic march seems the obvious course of action when trying to reduce the incidence of allergic diseases which is the aim of allergy prevention programmes.

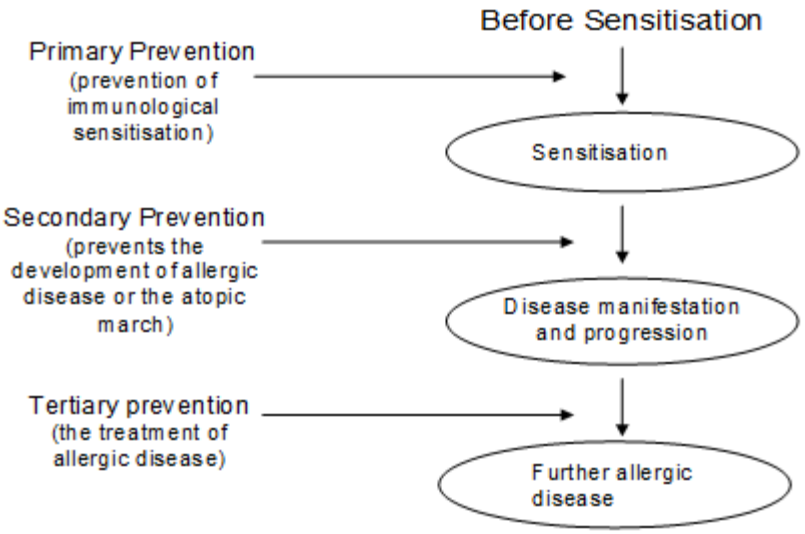

Figure 2.5 Algorithm of the 3 stages of allergy prevention. (71)

Most food allergy prevention strategies involve interventions related to maternal nutrition, breastfeeding practices, infant formula use and the nature of the infant diet. The rationale behind these strategies will be dealt with in turn.

2.7.1 Maternal Nutrition

It is well established that early-life factors influence the development of all atopic disease, including food allergy (72) with research showing that events taking place during pregnancy and foetal development play a key role in determining whether an infant will develop allergic disease (7;73-75). Nutritional factors affect the development and function of the immune system (76) and since the foetus is solely dependent on the mother for its nutrition it stands to reason that the maternal diet during pregnancy will affect the immune development of the foetus and therefore its likelihood to develop food allergy.

Maternal dietary factors that have been considered to have an impact on allergic outcome of the infant are polyunsaturated fatty acids, probiotics, vitamin, A, vitamin C, vitamin D, vitamin E, selenium, folate, zinc, fruit and vegetables, and dietary antigens (10;70). Those which have a more robust evidence base of involvement in allergy development with be dealt with in turn.

2.7.1.1 Polyunsaturated fatty acids

Intake of LCPufas has increased over the latter part of the twentieth century as a result of health concerns regarding the consumption of saturated fats (77) and this increase has been causally linked to the increase in allergic diseases (78;79). This

possible link is backed up by epidemiological observations (80-82) and is mechanistically explained by the fact that LCPufa's are precursors for eicosanoid inflammatory factors including prostaglandins (PG) and leukotrienes (LT). These Inflammatory factors are involved in modulating and controlling immune responses. Prostaglandins and Leukotrienes derived from n-6 Pufa's (e.g. sunflower oil) strongly promote inflammatory responses and play a role in allergic sensitisation (83) whereas the n-3 LCPufa's lead to production of the less biologically active prostaglandins and leukotrienes.

Intervention studies where the mothers diet has been supplemented with fish oils have been carried out (84;85) and whilst all demonstrated immunologic changes in cord blood, they could only suggest at improved clinical outcome (83). Further intervention studies are being carried out with details of clinical outcome at 6 months expected in the next year.

2.7.1.2 Probiotics

Intestinal microbiota plays an important role in the development of the mucosal and systemic immune system (86). It is an important stimulus for the development of the gut associated lymphoid tissue (GALT), the largest mass of lymphoid tissue in the body, and for the development of oral tolerance. Observational studies show differences in gut microbiota composition between atopic and non-atopic infants. Generally, atopic infants have less bifidobacteria and more clostridia than non-atopic infants. These differences precede the development of atopic disease since they already exist in the first few weeks of life, suggesting a causal relationship (87-89).

Manipulating the intestinal micro flora of atopic children towards a more "non-atopic flora" with probiotics, (which are live micro-organisms with various, strain specific immunomodulatory effects) could be a way to prevent allergic diseases. A number of double blind randomized placebo-controlled trials have been performed to investigate the ability of probiotics to prevent allergic disease in high risk infants but these studies have mainly focused on atopic dermatitis (AD) and not on food allergy and have conflicting results. In three studies the incidence of AD was significantly reduced (90-92). However, in the other study no effect on AD incidence was found (93). These conflicting results can possibly be explained by differences in study design, the use of different probiotic strains and dosages and/or genetic characteristics of the populations studied. Also all studies gave the probiotic to the infant after delivery in addition to the mother taking it whilst pregnant so any observed effect may be due to direct ingestion by the infant as opposed to via the maternal route. However, a recent study has shown an effect just on allergic disease with maternal supplementation (94).

2.7.1.3 Vitamin D

The role of vitamin D in allergy development was first proposed in 1999 (95) where its inhibitory effect on the production of the Th-1 cytokine IL-12 was highlighted and epidemiological observations such as the time course of the prevalence of increase in allergic diseases and vitamin D supplementation, and the clustering of disease in higher socio-economic groups (who are more likely to follow supplementation guidelines) were used to substantiate the argument. Since that date the role of vitamin D has been strongly debated and whilst the first papers advocated that increased vitamin D supplementation was the cause of rising allergy prevalence (96-98) due to its effect on IL-12, IL-2 and IFN-γ (99), others have since argued the protective effect of Vitamin D (100;101) due to its role in the development of regulatory T cells, and the proliferation of tolerising and anti-inflammatory cytokines such as IL-10 (102) .

The focus is not always on dietary vitamin D, the role of sunlight exposure (assessed by degrees latitude) has also been investigated but again there are conflicting results with some papers demonstrating high levels of sunlight (and therefore Vitamin D) to be inversely related to allergic disease (103;104) whereas others have shown the reverse (105;106).

As has been called for (65) there are currently intervention studies taking place looking particularly at the role of maternal D exposure and food allergy in their offspring to try and clarify the role of Vitamin D in allergy development.

2.7.1.4 Folate

The human body requires folate to synthesise, repair and methylate DNA (107). Consequently it has been a nutrient of interest in the growing field of epigenetics. Recent research has demonstrated that some epigenetic processes can influence the expression of Th_1, Th_2 and Treg cells (108)) and consequently resultant immune function.

Since folate supplementation is widely advocated for pregnant women to protect against neural-tube defects, it is possible that this advice may be leading to epigenetic changes in the offspring which may increase the risk of the child developing an allergic disease.

To date three studies have looked at the action of folate on the immune system (particularly allergic mechanisms). Two studies (one an animal study (108) and one using birth cohort data (109)) have shown a positive relationship between increased

folate intake and subsequent allergic disease. The third study used serum folate and total IgE levels from the National Health and Nutrition survey of the USA and demonstrated an inverse association (110). As is the case with vitamin D, further research studies have been urgently called for into the relationship between maternal folate intake and allergy development (70).

2.7.1.5 'Healthy diet'

The reason for the observed increase in the prevalence of asthma and allergic disease since the 1970's has been stated to be due to a change in diet, namely the decreased intake of fruit and vegetable intake (78;111) However, the studies looking at the 'healthfulness' of the diet have looked at different dietary constituents, have used different outcome measures and used different methodologies. This makes it difficult to interpret the findings in relation to the 'healthfulness' of maternal diet and allergy outcome. Willers et al (81) found an association between maternal fish and apple consumption and wheeze at 5 years whereas Chatzi et al (112) found an association between degree of adherence to a Mediterranean diet and wheeze and atopy at 6.5 years of age. However, Lange et al found no association between any maternal dietary pattern ('healthful' or not) and childhood wheeze (113). A meta-analysis carried out by Nurmatov (10) concluded that there was a (weak) protective effect of fruit and vegetable intake in the development of asthma and allergy and also a weak protective effect of a 'Mediterranean' diet for asthma outcome, but more research in this field is required.

2.7.1.6 Dietary antigens

It has been known for many years that cord blood contains immunological factors that demonstrate the foetus has been responding immunologically to its *in utero* environment (114). Consequently, it was hypothesised that if mothers eliminate dietary antigens from their diet whilst pregnant then it would reduce the risk of the child developing an allergic disease, since the antigens would not be present to cause inter-uterine sensitisation. The first randomised control trial to investigate this hypothesis was carried out by Falth-Magnusson et al (115), with a number of similar trials being carried out subsequently (12;116). None of these studies demonstrated a reduction in allergy risk associated with maternal allergen avoidance. However, these studies only intervened in the final trimester of pregnancy and since it has subsequently been demonstrated that cell proliferation in response to antigen stimulus could occur in the foetus as early as 23 weeks gestation (7) it was thought the negative results of these trials may be due to the fact that the avoidance interventions did not start early enough in pregnancy. Therefore an intervention study with maternal dietary exclusion of hen's egg from approximately 18 weeks gestation was carried out (117) but this did not demonstrate any real effect on the allergy outcome in the offspring

either. An additional complication in interpreting the results of all these studies was maternal compliance which was not quantified but was stated in one paper as being 'short of absolute' (12). Due to the inconclusive results of these intervention studies and concerns that allergen avoidance diets may adversely affect maternal nutrient status, allergen avoidance during pregnancy is no longer recommended as an allergy prevention measure (118-120).

2.7.2 Breastfeeding practices

It is widely accepted that breast milk is the food of choice for infants for a number of reasons such as cost, safety, psychological benefits and the prevention of infant disease such as gastroenteritis (121), wheeze (122), necrotising enterocolitis (123) and vomiting (124). Because of these undoubted health benefits of breastfeeding, studies comparing the effects of breastfeeding on allergy development are scarce (125). Two reviews of observational studies on the protective effect of breastfeeding have shown conflicting results. Muraro (9) reviewed 15 observational and 14 intervention studies and concluded that breastfeeding high risk infants for at least 4 months was associated with a reduced cumulative risk of cow's milk allergy until 18 months of age. Friedman and Zeiger (5) reviewed such studies and they highlighted that three studies published in the 1980's demonstrated that exclusively breastfed infants had lower serum IgE, less eczema and fewer asthmatic episodes but that four studies showed breastfeeding to have no protective effect on the development of food allergy. None of these studies were either randomised or prospective so there is a strong risk of bias in the results (a point emphasised by the authors). In 2001, Gdalevich et al performed two meta-analyses looking at the relationship between breastfeeding and eczema and asthma respectively (126;127) and found a significant protective effect against the development of eczema by breastfeeding. Breastfeeding for at least the first 3 months of life led to a decreased rate of asthma in high risk infants. However, a 2004 systematic review (128) concluded that at least 6 months of exclusive breast feeding did not protect against the development of food allergy by the age of 1 year when compared to infants who were exclusively breastfed for 4-6 months and this work has been supported more recently, by Kramer and colleagues (129) who performed a cluster randomised trial comparing infants from centres randomized to an intervention promoting breastfeeding to those not receiving this intervention. After the experimental intervention, exclusive breastfeeding rates at 3 months were 44.3% compared to 6.4% in the control group who did not receive any breastfeeding advice. At follow-up at age 6.5y, no differences were seen in the prevalence of asthma, atopic dermatitis, allergic rhinitis, or skin test positivity to common inhalant allergens (129).

Most of the studies that examined the effect of breastfeeding on food allergy were carried out in unselected cohorts with regard to allergy risk (130-136). Only two were able to demonstrate a protective effect of breastfeeding on food allergy (134;137). Relatively few studies have assessed the effect of breastfeeding in high risk infants (134;138;139). One of these (non-randomized) studies reported a prevalence of cow's milk allergy at age 1.5 years of 3.6% in breastfed infants or infants fed hydrolysed infant formula, as opposed to 20% in those not receiving preventive dietary measures (138). Recently, a long term follow-up study of high risk infants examined the role of breastfeeding on a number on allergic outcomes, including food allergy (140). This study showed that while there was a modest protective effect of breastfeeding on food allergy (as well as asthma, atopic dermatitis and allergic rhinitis) up to the age of 7 years, this risk was paradoxically increased after the age of 7. Additionally the German Infant Nutrition Intervention (GINI) study (141) seems to show that any benefit provided by exclusive breastfeeding appears to be conveyed only in high risk infants.

In summary, although it is widely agreed that breast milk is the food of choice for infants, its role in allergy development remains unclear with different studies showing, protection, no effect and even increased risk. This may be due to variations in breast milk composition and/or differences in maternal diet (85;142-144), as well as a lack of precision in the way infant feeding has been measured. Although it would be desirable to have studies that directly assess the effect of breastfeeding on the development of allergic disease, there are both methodological and ethical problems with conducting such trials which will probably continue to limit the quality of the evidence supporting the role of breastfeeding in the prevention of allergic disorders.

2.7.3 Role of infant formula in allergy prevention

It has long been agreed that feeding with a whole protein infant formula compared to breastfeeding increases the risk of the child developing allergic disease. (145;146). However, there will always be a demand for some form of alternative feeding choice for mothers who cannot or choose not to breastfeed so it is important that the risk of allergy development associated with infant formula use should be reduced. This has been achieved by using an alternate protein source to cow's milk such as soya or by the modification of the cow's milk protein in the infant formula by hydrolysis to reduce its allergenicity.

2.7.3.1 Soya formula

The use of soya-based infant formula differs geographically and there has been a debate associated around its use and whether it leads to increased likelihood of allergy to soya (147-149). However, a meta-analysis carried out in 2004 (150) concluded that

soya formula should not be recommended for the treatment of milk allergy in infants and in the UK the chief medical officer stated that soya formula should not be given to infants under the age of 6 months (151). More recently, the American Academy of Pediatrics stated soya formula should not be used for the prevention of food allergy (118).

2.7.3.2 Hydrolysed infant formula

There have been a number of studies examining their merits as a primary prevention for allergic disease. Intervention studies that have been carried out in high risk infants who are not breast fed have compared both partially and extensively hydrolysed cow's milk formulas to formulas based on intact cow's milk. Cow's milk allergy has been the principle outcome of interest as ascertained by both open and double blind challenge tests. In two studies, a protective effect of extensively and partially hydrolysed infant formulas was found (152;153) while in three other studies, no difference was seen (154) (32;155). One study included follow-up to age 7, at which time point no differences were noted (156). The GINI study examined three different hydrolysed infant formulas and standard infant formula, comparing different allergy outcomes between the groups. Both the extensively hydrolysed casein formula and partially hydrolysed whey formula were shown to have a preventative effect on both physician diagnosis of allergic manifestation and atopic dermatitis. Inexplicably, this was not found for the extensively hydrolysed whey formula (157). It is because of these somewhat contradictory findings that recommendations may not specify whether an extensively or partially hydrolysed formula should be used but that it should be of "proven reduced allergenicity" (119). It should be noted that the GINI study was designed to compare the allergy preventive effects of hydrolysed formulas as compared to standard infant formula, not breast feeding.

Despite the fact that these studies have often been conducted as randomised, controlled trials, they have frequently been characterised by a number of methodological shortcomings, including problems with blinding, inadequate outcome assessments, and inability to distinguish true prevention from delaying symptoms (158). However, a Cochrane review on this topic conducted in 2006 (159) concluded that 'there is no evidence to support feeding with a hydrolysed formula for the prevention of allergy compared to exclusive breastfeeding. In high risk infants who are unable to be exclusively breastfed, there is limited evidence that prolonged feeding with a hydrolysed formula compared to a cow's milk formula reduces infant and childhood allergy and infant cow's milk allergy.'

2.7.3.3 Inclusion of pre/probiotic in infant formula

In the endeavour of infant formula companies to further reduce the allergenicity of their infant formulas and make their composition more comparable to breast milk, they have started to include pre and/or probiotics into their infant formulae.

A number of double blind randomized placebo-controlled trials have been performed to investigate the ability of probiotics and prebiotics to prevent allergic disease in high risk infants. These studies have mainly focused on AD and not on food allergy. The five studies with probiotics show conflicting results. In three studies the incidence of AD was significantly reduced (90-92). However, in two other studies no effect on AD incidence was found (93;160). Also, the *Bifidobacterium* strain used in the trial of Wickens et al did not reduce the incidence of Atopic Dermatitis (92) Two studies included prevention of cow's milk allergy or food allergy as an outcome and found no effect (90). These conflicting results can possibly be explained by differences in study design, the use of different probiotic strains and dosages and/or genetic characteristics of the populations studied. In none of the studies a reduction in the incidence of other allergic diseases, such as asthma or allergic rhinoconjunctivitis, could be demonstrated and one study even showed an increase in the incidence of wheezing bronchitis in the probiotic group (93). Currently, only one prevention study with prebiotics has been performed. This study showed a significant decrease in AD incidence (161) and at age two there were significantly less children with recurrent wheeze or allergic urticaria in the prebiotic group (162). Unfortunately, sensitization was not included as an outcome measure.

Meta-analyses into the role of pre and pro-biotics on allergy have been carried out and in both cases the conclusion was that there was currently insufficient evidence to determine their role in allergy prevention and further trials were needed (163;164).

2.7.4 Nature of the infant diet

2.7.4.1 Infant feeding policies

The progress of a child from a full milk diet (whether that is breast milk, breast milk and infant formula or wholly infant formula) to a diet consisting of solid foods is by its very nature an essential process that every child has had to conquer successfully and as infant mortality rates demonstrate, our ability to bring infants through this process has improved greatly throughout the 20th Century. In 1900 the UK infant mortality rate was 140 per thousand live births, dropping to 22.5 per thousand live births in 1960 (165) and it is currently 4.62 per thousand live births (166). Whilst this improvement is due to progress in general public health measures, advances in nutritional knowledge which have led to improvements in infant feeding product's

(infant formula and complementary feeding products) and practices (167) have also played a role. Despite these advances, further improvements to infant health and mortality are still searched for, leading to regular changes in infant feeding guidelines. Policy makers need to be aware that infant feeding practices set the foundation for later health, so actions that appear to be beneficial in the short term may be storing up future health problems (168). Consequently, before we make changes to existing policy, we need to make use of the findings of any systematic reviews in the field as well as being aware of past and current feeding practices and corresponding health data to enable the effect of any change in feeding policy to be assessed. We must endeavour to do "no harm" so any undesirable health consequences need to be identified quickly and any necessary policy changes are dealt with in a timely fashion. Examples of such processes taking place is the Committee on Toxicity of Chemicals in food (COT) advice for peanut consumption during pregnancy and lactation issued in 1998 (169) being amended in 2008 as a result of new evidence (120) and the ideal age for the introduction of gluten into an infant's diet which was recently reviewed jointly by COT and Scientific Advisory Committee on Nutrition (SACN) (170) after a statement issued by the European Food Safety Authority (EFSA) in 2010 (171).

2.7.4.2 Recommended age for solid introduction

In 2003 the WHO advised exclusive breastfeeding for up to 6 months (23) and this advice was backed up by the Department of Health (DoH) (172). Prior to this the advice was for solids to be introduced between 4-6 months (but not before 17 weeks) (24). The necessity for exclusive breastfeeding for 26 weeks has recently been called into question and the ideal age for solid introduction is currently being looked at by SACN. However, despite the current recommendation, less than 1% of mothers achieve the 26 week exclusivity target (173) with recent data (174) demonstrating that exclusivity is lost due to the introduction of infant formula in 81% of mothers.

In addition to the advice given to mothers regarding ideal age for solid introduction, the advice also recommended delaying the introduction of allergenic foods beyond 6 months (175), with other countries until recently recommending further delaying the introduction of allergenic foods even further (15). Worryingly, this advice was given despite there being little research at the time looking solely at the relationship between solid food introduction and the later development of allergic disease. Fergusson et al looked at both the timing and rate of introduction of solids into an infant diet with the later development of eczema at two years (176) and found that solid food introduction before 4 months of age and the number of foods introduced was associated with an increased incidence of physician-reported eczema. This association persisted to 10 years of age when a range of confounding factors including family history of atopic disease, other infant diet factors and family socio-

economic status where also included in the analysis (177). Also in the 1980's, Kajosaari compared the introduction of allergenic foods early (at three months) as compared to after six months of age and showed no differences in the prevalence of food allergy when outcome was assessed by double blind challenge, either in the first year of life (178) or at age five (179). Other studies have looked at the relationship between timing of complementary feeding and allergy development but differences in methodology makes it difficult to draw firm conclusions. A systematic review looking at complementary feeding before 4 months of age could find little data linking early solid feeding and allergic conditions other than an association with early solid introduction and persistent eczema (180). However, many of the studies reviewed lacked a rigorous design and were subject to recall bias, did not incorporate confounding factors in the study design and used less than ideal diagnostic criteria.

From 2004 onwards, infant feeding data collected as part of birth cohort studies have been analysed to investigate the relationship between solid food introduction and the later development of atopy (181-184). No study found any benefit on allergic outcome by delaying the introduction of solids and two found an association between the delayed introduction of milk (183) and egg (182;184) and increased incidence of eczema and atopic sensitisation. More recently it has been suggested that children exposed to cereal grains before 6 months of age (as opposed to after 6 months of age) are protected from the development of wheat-specific IgE (185). However, all studies collected feeding data retrospectively which makes the findings vulnerable to both recall bias and reverse causality.

Nevertheless, these studies have raised the possibility that delaying the introduction of foods into an infant's diet (particularly delaying the introduction of allergenic foods) is not beneficial and may actually increase the risk of the child developing allergic diseases as suggested by a number of authors (181-186).

Further research where infant feeding data are collected prospectively is needed to establish what effect delaying the introduction of solids in general and allergenic foods in particular into the infant diet has on allergic disease (65).

2.7.4.3 Specific nutrients and allergy development

A number of nutrients are known to have an immunological role. These include polyunsaturated fatty acids, anti-oxidant vitamins (vitamins A, C, E and β-carotene), vitamin D, iron, selenium, zinc and folate (76) (187) (102) (188). The potential role of these nutrients in the aetiology of allergy have been highlighted in several review papers (10;65;70;189) but the studies included in these reviews are generally observational with high heterogeneity, some use serum levels of the nutrient as a

measure of dietary intake whereas others use retrospective dietary assessment. Additionally most studies looking at dietary intake and allergy development have focussed on the timing of solid introduction (in particular of allergenic foods), few have concentrated on nutrient intake. Of those that have looked at nutrient intake, they looked at current diet and disease status (190;191), not the nutritional profile of the infant diet and the later development of allergic disease.

However, of the studies that have looked at the timing of specific allergenic foods into the infant diet and allergy outcome, the observed effects may be due to differing dietary nutrient profiles resulting from the inclusion or avoidance of certain foods, but this has not been looked at. For example, two papers have reported an association between the delayed introduction of egg into an infant's diet and increased allergy risk (181;192) however this association may not be due to the timing of egg into the diet *per se* but the fact that delaying the introduction of egg into the diet may lead to a diet with lower levels of the immunologically active nutrients of vitamin A, vitamin D, vitamin E, zinc and selenium. Additionally, Alm et al (193) and Kull et al (194) reported from Sweden on an association between delayed fish introduction into an infant's diet and allergy outcome. Again, this association may not be due to delaying the introduction of the allergenic food fish into the diet but be due to a reduced intake of Lcpufa's, selenium, zinc and vitamin D.

Consequently, investigation into the nutrient profile of an infant's diet in relation to allergy outcome needs to be undertaken to establish if there is any relationship between the nutrient intake of infants and allergy outcome.

2.7.4.4 Infant feeding patterns and allergy development

Analysis of dietary intake by looking at the pattern of dietary intake as opposed to focussing on individual dietary components has become popular and has been advocated as a valid method of looking at nutritional data. Its advantages are that it can take into account nutrient interactions of known or unknown effects which is thought to be particularly useful when looking at disease aetiology.

More recently dietary pattern analysis has been used for the analysis of infant diet either as a descriptive technique (33;195) or related to clinical outcome (196;197). To date there has only been one publication using pattern analysis and outcome that may be associated with allergy (113). This study looked at the maternal diet in the first and second trimester of pregnancy and outcome data of the infant at three years of age (recurrent childhood wheeze). To date there has been no published work looking solely at dietary pattern analysis in infants and allergy outcome. Since the aetiology of allergic disease appears to be so complex, pattern analysis may be able to identify

feeding patterns that are associated with the development of food allergy or that may be protective against the development of food allergy.

3. Rationale for and explanation of methodologies used

3.1 Definition and categorisation of food allergic (symptomatic) infants and their controls

The outcome measure of any research needs to be well defined. Since this thesis is looking at factors affecting the development of food allergy by the end of two years of age, what is referred to as food allergy (the outcome measure of this research) needs to be clearly understood.

In the literature, food allergy can be diagnostically defined in a number of ways. First, is perceived food allergy where the patient (or parent when the reaction occurs in children) believes they (or their child) have clinical symptoms resulting from eating a certain food but have had no medical tests to confirm their opinion. This is also called self-reported food allergy. A second commonly used diagnostic criterion is the presence of specific IgE detected either by skin prick or blood test. However, since not all food allergy reactions involve IgE, this measure can lead to a false negative diagnosis of food allergy. Finally, food allergy can be diagnosed by the process of food challenge (4).This is where the food is removed from the diet and then re-introduced in a controlled manner. If symptoms improve after elimination of the suspected food then it is reintroduced to the diet; if symptoms return it is considered the suspected food is causing clinical symptoms and a positive diagnosis is made. Both steps of this challenge need to be completed as clinical symptoms can resolve spontaneously and therefore recovery from symptoms on removal of the food from the diet may not be due to the removal of the food from the diet so the reintroduction of the suspect food is key to an accurate diagnosis.

There are a number of different food challenge methods. Firstly, there is the 'open challenge' where everyone involved in the procedure knows that the suspected food is being introduced into the diet. Secondly there is the 'single blind challenge' where the challenge takes place in two part; one part has the suspected food hidden in another food/dish which is known to be tolerated; and the second part when just the alternative food is given. The overseeing clinical team know which part is which but the subject of the challenge does not. However, the gold standard in food allergy diagnosis is the double blind placebo controlled food challenge (DBPCFC) (198). This has the same procedure as for a single blind challenge except in this case neither the

participant nor the overseeing clinical team know which part contains the suspected food and which does not. After both parts of the challenge have been completed, the challenge is decoded to determine which part contained the suspected food (the 'active' part) and which did not (the 'placebo' part) (199). The main strength of this procedure is it broadly eliminates measurement and reporting bias in the clinical team and psychological reactions from the participant, leading to a valid diagnosis (200).

In this study the outcome measure was food allergy as determined by DBPCFC. Since the infant either had a positive or negative DBPCFC, the outcome measurement was categorical.

The control infants did not have a food allergy. They were assessed using the same criteria as an infant suspected of having a food allergy and had no reactions that may be attributable to food. By definition, they also had a serum specific IgE level below the diagnostic cut-off of 0.35kUl/L.

3.2 Rationale for the determination of study hypotheses

It is important that study hypotheses are focused and precise so that study outcomes can be effectively and accurately interpreted.

3.2.1 Hypothesis 1[Footnote]

The first area of interest for this study was the nature of the relationship between feeding with breast milk and the later development of food allergy. There are four aspects of breastfeeding that may play a role in allergy development: initiation, duration, duration of exclusive breast feeding, and 'overlap feeding' between breast milk and the common food allergens. As a consequence, it was decided to split this first hypothesis into four parts.

Hypothesis 1:

1a. Children who develop food allergy by two years of age will have a 5% lower rate for the initiation of breast feeding than children who do not develop a food allergy.

1b. Mean breastfeeding duration for children who develop food allergy by two years of age will be 4 weeks less than for children who do not develop a food allergy.

1c. Mean exclusive breastfeeding duration for children who develop food allergy by two years will be 4 weeks less than for children who do not develop a food allergy.

1d. Mean duration of concurrent breastfeeding with any cows milk protein for children who develop food allergy by two years will be 4 weeks less than for children who do not develop a food allergy.

As regards breastfeeding initiation (hypothesis 1a), in 2005 the breastfeeding initiation rate for England and Wales was 77%, but there was a 22% difference in breastfeeding initiation between mothers of differing socio-economic classification, and 17% difference between mothers of different educational achievement (173). Since it is possible to have differing breastfeeding initiation rates within sub-populations of study populations, there could be differing feeding rates within the thesis population which relate to allergy outcome. Therefore, hypothesis 1a specified a 5% difference in breastfeeding initiation rates between the groups.

The infant feeding study of 2000 and 2005 showed no increase in breastfeeding duration for England and Wales (173). The mean breastfeeding duration for England and Wales in 2005 was 6.4 weeks (value calculated from raw IFS data) but the range was between 0 and 15 weeks with a standard deviation of 3.334 showing great variability in duration. Consequently, a difference of breastfeeding duration of 4 weeks between the study groups was thought plausible and was specified for hypothesis 1b. Similarly, for exclusive breastfeeding (hypothesis 1c), since the IFS raw data showed the variance for exclusive breastfeeding to be similar as that for breastfeeding duration.

For concurrent breastfeeding, general UK weaning advice is not to introduce allergenic foods (eg egg, wheat, fish) until after 6 months of age (201) and the 2005 IFS data shows breastfeeding generally did not continue until this age. Consequently, the most likely concurrent feeding is with Cows' milk protein which is generally introduced into the infant diet before 26 weeks of age. However, there is no published data regarding duration of concurrent breastfeeding so the difference between the groups of 4 weeks used for hypotheses 1b and 1c was also used for hypothesis 1d.

3.2.2 Hypothesis 2[Footnote]

Findings from the IFS show the mean age solid introduction for mothers from England and Wales was 19 weeks (173) and that 31% of mothers stop breastfeeding between 17 and 22 weeks of age. There is 4 weeks variation in the mean age of solid introduction according to a number of population demographics (maternal age, socio-economic classification and education). As was the case for breastfeeding, this demonstrates there can be variation in feeding practices within study populations. Consequently, a mean difference of 4 weeks for the age solids were introduced into the infant diet was thought plausible and was specified for hypothesis 2.

Hypoyhesis 2: Children who develop food allergy by two years of age will have solid foods introduced into their diet a mean of 4 weeks earlier than children who do not develop a food allergy.

3.2.3 Hypothesis 3[Footnote]

A number of nutrients are known to be involved in immunological/allergy mechanisms. These include protein, fat, iron, zinc, selenium, and vitamins A, C and E (see section 2.7.4.3). As there is no raw data available for the levels of dietary intake of these macro and micronutrients for healthy infants, a difference of 0.5 of standard deviation between the groups was decided upon as an indicator of variation in intake which may cause differing immunological outcomes between the groups.

3.2.4 Hypothesis 4[Footnote]

The relationship between infant dietary patterns and health outcomes is a relatively new area of research. Previous studies have used the difference in standard deviation in the outcome measure to interpret the results and give them clinical relevance (197). This isn't possible for this study as the outcome measure is categorical. However, it is possible to use the difference in the standard deviation of the patterns score between the outcome groups. Consequently a difference of 0.5 of a standard deviation of a pattern score between the groups was decided upon for hypothesis 4.

3.3 Rationale for dietary assessment and analysis techniques used

The method used to assess dietary intake needs to be matched with the necessary level of accuracy (be fit for purpose), needs to be deliverable from the methodology and should not be so burdensome to complete as to affect compliance. Also, since all methods have errors, a particular challenge is minimising random errors while trying to avoid systematic errors (bias)(16).

The following sections of this chapter will review methods that may be suitable for use in the present study. To do this the requirements of dietary data collected to meet the study objectives first need to be considered.

3.3.1 Data requirements

The dietary intake data collected:

Hypothesis 3: Children who develop food allergy by two years of age will have a 0.5 standard deviation difference between the mean intake of certain macro and mico-nutrients. Nutrients included in the analysis are energy, protein, fat, iron, zinc, selenium, vitamin A, vitamin C and vitamin E than children who do not develop a food allergy.

Hypothesis 4: Children who develop food allergy by two years of age will have a 0.5 standard deviation difference in their PCA pattern score than children who do not develop a food allergy.

- needed to be 'raw', that is original and unadulterated so it provided complete data that could be coded in a number of different ways to allow the resultant variables to be analysed in a number of different ways;
- needed to reflect the inherent variability of the infant diet in the first year and the ever expanding variety of foods taken once solids had been introduced;
- needed to capture 'one off' events (such as grandma giving the infant chocolate as a treat!!);
- needed to provide some quantitative data to allow nutrient intake levels to be calculated.

Additionally the method chosen to meet the above criteria needed to not be so burdensome that initial parental agreement to keep the diary and on-going compliance in completing it were adversely affected, whilst at the same time providing enough data to minimise bias.

3.3.1.1 Choosing a dietary assessment method

3.3.1.1.1 General principles

Since different dietary assessment techniques result in diverse types of data it is essential the correct assessment technique is used for the required purpose. If details of micronutrient intake in a population group are required, the technique used would normally differ from that needed to investigate food pattern intake within the same population group. Similarly, although a food record is considered the gold standard, if the data required is for usual intake then a food frequency questionnaire (FFQ) may provide a more accurate representation; since the food record may not have been completed for long enough to deliver an accurate representation of usual intake (16). In addition to what data is required, other considerations to make when choosing dietary assessment method should include resources available (i.e. staffing levels available to collect and analyse data), the likelihood the population of interest will be able to fulfil the requirements of dietary completion and practicalities as to how the data will be collected.

3.3.1.1.2 Selection of dietary assessment technique for this research project

Ortiz-Andrellucchi commented that dietary assessment in infants is difficult as their dietary habits are rapidly changing (202); unfortunately, it was this particular element of an infant's diet that was of interest in this research. As a consequence, a methodology that best captured this aspect of the infant diet had to be selected.

A FFQ could have been administered every few months to provide data on how the infant diet changes over time whilst minimizing the degree of dietary recall required. A systematic review of previous studies looking at dietary intake in infants revealed they all used specifically designed FFQ's (202) and all validated their methodology satisfactorily. It followed that an FFQ is considered an appropriate measure for this population group. However, for this particular study, the dietary elements that may subsequently be shown to be protective (or vice versa) in the development of allergic disease were not thoroughly known so administering an FFQ may not capture the dietary data required to investigate any potentially causative or protective feeding practises.

It was considered the only way an infant's diet could be seen as a whole (so capturing its rapidly changing nature) in enough detail to answer the objectives of this study was to request the mothers/carers to record all the infant ate or drank for the first year of life. This is a massive task to ask, but bearing in mind many mothers/carers already record what their infants eat for the first few months of their infant's life (particularly when introducing solids foods) as this is recommended by a number of infant feeding books (203;204), it was believed this existing behaviour could be captured and built upon for the study. Additionally, keeping the amount of detail required to a minimum was considered a way to reduce the effort required to complete the diaries thereby encouraging participation.

Consequently it was decided to ask mothers to keep a food menu record for three consecutive weeks followed by a fourth week where quantitative data was recorded in addition. To obtain the co-operation of the mothers/carers completing the dietary records, a face-to-face explanation of how to keep the food records was given along with encouragement and support including regular up-dates on study progress as this has been shown to aid compliance (205).

3.3.2 Choosing dietary assessment analysis techniques

3.3.2.1 General principles

Data derived from a food diary can be considered as 'raw' as it is a record of what has been eaten or drank with no categorisation of the data which can reduce detail. As a consequence, this raw data can be processed and analysed in a number of different ways to achieve different resultant variables. However, to best utilise this form of data it first needs to be appropriately categorised/coded. The data can be used to describe the whole study population or the population can be categorised according to demographic or outcome variables and the data compared between the different categories.

Dietary data can be described using a number of different statistical techniques. Descriptive statistics can give mean (median when the data is not normally distributed) values for nutrient intakes with categorical data being analysed to describe whether certain foods are eaten or not. These values can be plotted against time to demonstrate how intake changes with time.

Survival analyses can be used to describe how intake increases or decreases over time and pattern analyses can be used to describe the diet as a whole. All of these tests can be used to compare differences between study groups. Correlation and regression analyses can be used to investigate the relationships between the study groups and or variables.

3.3.2.2 Selection of dietary assessment analysis techniques for this research project

To meet the study objectives a number of techniques were used to code and analyse the data.

For objectives 4 and 5[Footnote] the infant food diaries were used to provide a categorical coding of the presence or not of the food of interest. Although coding of the presence of foods per day would be the most complete coding of available dietary data, 365 time-points for each food of interest for the whole cohort would lead to an unwieldy database. Additionally, infant feeding recommendations and other infant feeding publications do not use days as the unit of measure so for practicality and comparability issues, a decision was made to code the data weekly.

The foods of interest were decided by consideration of what infant feeding recommendations were applicable to this age group (24;119;172;206), what foods previous infant feeding publications had investigated (173;207;208), and what foods had been cited as being of interest in the development of food allergy (27;141;181;184;192;194;195). 107 food category variables were decided upon and these are detailed in Appendix 2. Using this coding methodology, each infant had a yes/no picture of which of these 107 variables were present in its diet for each week a food diary was completed. From this information the age the infant first ate the food of interest, how often it ate it and for how long it was present in the diet could be

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Objective 4= Use of prospective food diary data to determine the nature of the weaning diet for children diagnosed with food allergy by the age of 2 years and their two-age matched controls.
Objective 5= Use of prospective food diary data to identify dietary patterns in children diagnosed with food allergy by the age of 2 years and their two-age matched controls.

calculated using a SAS version 9.2 generated computer programme designed for the purpose. Further analysis of these SAS derived variables allowed follow-on investigation of infant feeding variables such as duration of breastfeeding, age at solid introduction, nature of concurrent breast feeding and observed infant feeding patterns. These variables could then be further compared between the infants diagnosed with a food allergy (symptomatic) and their controls by tests such as Chi-square, t-tests and Mann-Whitney U test.

In addition to these common statistical techniques, further statistical techniques were used to further explore the infant feeding practices that may be associated with food allergy development. The first of these was Kaplan Meier survival analyses which allowed for analysis of breastfeeding duration and cessation, and solid introduction habits.

Another analysis used to interpret the newly derived variables from the SAS analysis was Principal Component Analysis (PCA). This is a type of whole diet analysis that enables dietary patterns to be described at a population level (209). Whole diet analysis is being increasingly used as it is becoming apparent that dietary patterns often effect health outcomes as opposed to distinct dietary components or nutrients (210). This type of analysis can identify aspects of the infant diet that are related to health outcomes (197) and such an approach was adopted by Zutavern in her work looking at solid food introduction and the development of asthma and eczema (181) but only to identify variables of interest to incorporate into a multivariate analysis.

PCA is a method that reduces an original set of variables (such as timing of solid introduction, duration of breastfeeding etc.) into a smaller set of uncorrelated components that represent most of the information found in the original variables. It produces the uncorrelated components by aggregation of food groups according to the degree they correlate with each other (211). The first principal component accounts for the maximal amount of variation, the second component accounts for the maximal amount of the variation that remains after the first component, and so on until all the variation is accounted for. Within each component the contribution that each of the original variables makes to the variation can be expressed to get a sense of which of these variables are explaining most of the variation within the study population. Each derived principal component can be treated as a new independent variable, with each subject in the study having a score for that component which can then be used in further statistical tests to see if the dietary patterns they describe are associated with clinical outcome (which in this case is the development of food allergy).

For Objective 3[Footnote] nutrient intakes across different time points for the symptomatic and control infants needed to be determined. This data was then compared to establish if there was a difference in the intake of the nutrient of interest between the groups. This required a repeated measures analysis of variance (RM-ANOVA) as the same subjects provided the data at each time-point. If a one-way analysis of Variance (ANOVA) had been used then the data would be subject to a type I error (a likelihood that an effect is shown when there is not really one present) since multiple comparisons on the same subjects would have been made. A RM-ANOVA takes multiple comparisons into account in its design and so reduces the likelihood of a type-1 error being committed.

Finally a logistic regression analysis was carried out to establish which of the variables shown to have an association with food allergy outcome maintained the association when the effects of other variables were controlled for. Logistic regression analysis also enables calculation of the relative risk of each variable on allergy outcome allowing the effect of any change in behaviour to be established.

3.4 Validity, reliability and sources of error in dietary assessment and analysis

3.4.1 General principles

Research results can be considered to be valid if they are a reasonable representation of the true situation (212). Whilst this is difficult in any research field, it is particularly difficult to collect data that reflects true dietary intake and it is now recognised that errors are inherent in any dietary assessment method (213). Consequently it is important that the relationship between the data collected and the true picture is considered and where possible quantified. This process is called validation.

Until the 1980s, because of the lack of a suitable external standard against which dietary intake could be measured, a method was judged acceptable if the mean intake as measured by two different methods did not significantly differ and if correlations for nutrient intakes between individuals exceeded 0.5 (205). However, in the 1980s researchers started using biochemical techniques to validate dietary intake (214;215) and in 1987 Bingham defined a biological marker as “any biochemical index in an easily accessible biological sample that in health gives a predictive response to a given

Objective 3= Determination of mean daily nutrient intake data from quantitative prospective food diary data for infants diagnosed with food allergy and their two-age matched controls.

dietary component" (216). Currently there are good biological markers for energy and protein but reliable biomarkers for more nutrients are still required (217). However, whilst the use of biomarkers for validation can be useful, it needs to be remembered that blood concentration of micronutrients may reflect individual differences in metabolism and absorption as well as dietary intake (202).

The use of urinary nitrogen and doubly labelled water (DLW) methods to validate dietary intake demonstrated that self-reported energy intakes were often implausible due to under-reporting. However, due to the expense of these methods, another technique is used to validate dietary assessment techniques where the reported energy intake is compared to the expected energy expenditure requirements. This is called the Goldberg equation and a paper by Black explains its use in the critical evaluation of energy intake (218).

Reliability is a measure of the ability of a data collection technique to produce consistent results when the same variable is measured under the same conditions (219). However, because the diet of every individual varies on a weekly, monthly and seasonal basis (213), it is difficult to assess the reliability of a dietary assessment method as the diet will not be exactly the same at two different time-points. Add to this the change in dietary intake that occurs for infants in their first year of life, then determining the reliability of a dietary assessment measure for the infant diet becomes very difficult.

All dietary assessment methodologies are subject to sources of error to some extent or another. There are four major sources of error: sampling bias, response bias, coding error, and use of food composition tables (216). In addition to these study-wide consistent errors, there are measurement errors associated with specific methods of assessment and are subject to participant variation. These measurement errors are: estimation of portion size, recall or memory error, day-to-day variation intake, and the effect of the survey method on intake (205).

3.4.2 Consideration of reliability and sources of error for this study

As this is a case control study, the major consideration is that any sources of error present can be considered to be having an equal effect in both experimental groups.

Since the infant diet is changing constantly (202), the most common method of assessing reliability of a dietary intake assessment method (test-retest (219)), cannot be used. However, food diary records for older children and adults are considered to be generally reliable, and the weighed food diary is considered the gold standard of

dietary assessment (220) which implies the reliability of a food diary as a dietary assessment technique is acceptable. Reliability may differ between the experimental groups with parents/carers of symptomatic infants changing how they record data as a result of their infant's food allergy. Nevertheless, since all the analyses in this study except that of 'on-going infant diet patterns' uses data collected before diagnosis, then this potential for difference between the experimental groups can be considered to be minimal.

For coding errors and errors associated with the use of food composition tables, the same protocol was used for each study group so any error should be the same for both groups and for all analyses except the nutrient composition of the infant diet. Furthermore, the same researcher carried out all the data processing, coding and analysis thereby reducing the likelihood of there being a difference between the two study groups. However, for the analysis of the quantitative data, three researchers coded the diet data and this is a potential source of error. To minimise this risk, a strict protocol for coding the dietary intake data and for using the food composition tables was used. To further minimise this source of error, the same database (which included data added in for this project) was used by all researchers. To identify the extent of any inter-observer error, 10 infant diaries were coded by each researcher and the results compared.

Response bias has been shown to be affected by knowledge of the participant on how to complete the dietary record as well as a desire to give the 'correct' answer. Errors resulting from response bias can be minimized by fully explaining to participants what data is required and that there is no 'correct' answer (16); this was explained to all mothers participating in this study. Some response bias may be affected by background demographics so reducing differences between experimental groups where possible can reduce the effect of response bias in case control studies. In this study, controls could not be selected by their demographic background to minimise differences with the symptomatic infants, but background differences could be investigated to determine if there was any demographic differences between the groups which may be a source of error. If such differences existed, they could be controlled for (where possible) in the analyses carried out.

The errors that are subject to participant variation are of course equally applicable to both study groups. To minimise the effect of portion estimation/measurement, all mothers were advised on how to use household measures to complete the food diary and, as quantitative data was only collected one week out of four and only used for the nutrient analysis, the error resulting from portion size estimation has been kept to a minimum. Mothers were also encouraged to write down

what the infant ate at the time in order to minimise recall error and they were encouraged to keep the food diary close at hand to facilitate this. Error as a result of dietary variation is not such an issue when daily food diaries are completed prospectively.

It can be considered that some demographic characteristics may affect a participant's response and so could be a cause of variability in responses between participants. As was the case for reducing potential response error, instruction as to the importance of collecting accurate data for the analysis was highlighted when mothers were instructed on how to complete the food diary.

Despite all efforts taken to reduce potential errors in this study's data, all sources of error cannot be eliminated so the data cannot be considered to be 100% accurate. However, since this is a case control study, if any differences between the study groups are kept to a minimum for all confounding factors and potential causes of error, then differences seen between the groups for the measures of interest can be considered to be due to true differences in the data collected.

Additionally, if the sample population does not represent the general population (i.e. there is a sampling bias), that is not a source of error in this study unless the demographics of each study group are different from each other. However, if the study population is significantly different from the general population then any study findings should be extrapolated to the general population with care.

3.4.3 Selection of dietary validation technique for this study

In order for the intake data from the menu/food records collected in this study to be interpreted accurately, it needs to be demonstrated they are indeed an accurate measure of dietary intake of participant infants. Since the validation of any dietary method (including food records) requires comparison of the intake data with one or more objective measure (16), it needs to be determined what objective measures can be used to validate the diet records collected.

The cost of both the DLW and urinary nitrogen methods ruled these out as possible validation techniques in this research project and the Goldberg equation has not been demonstrated as applicable as a validation technique for infant energy intake so was not used.

Collection of additional intake data for each infant could provide an indication of the validity of the data collected, but obtaining such information was difficult. Asking another member of the infants' family to complete an additional diary on a number of

different occasions for comparison with the primary food diary would provide the required data, but collecting it may reduce compliance in the primary diet recorder since they may not appreciate the implication that their diary recording techniques need substantiating. Alternatively, asking parents/carers to take a photo of the food offered and then another photo of the leftover food could again be used as a source of validating intake data but collecting and delivering this data to the study team may prove onerous and also effect overall compliance with completing the food diary. Consequently, neither of these potential methods was used. Instead the dietary data collected in this study will be validated to some extent using the age appropriate estimated energy requirements (221) and also data from the 2005 infant feeding study (173).

4. Methodology

4.1 PIFA study background and methodology

4.1.1 Background

The thesis study design is a 'nested within a cohort, case control' study with the subjects being infants from the PIFA study who had been diagnosed as having a food allergy by DBPCFC by the age of 2 and their two age-matched controls.

The PIFA study, which acted as the parent study to this thesis, was a prospective birth cohort of 1170 babies recruited between 2006 and 2008. It was one of the 9 national birth cohorts that made up the complete birth cohort of the EuroPrevall project, a multidisciplinary project involving 53 partners with an EU contribution of 14.5 Million Euros (9.6 Million Sterling). All infants were followed through to 2 years of age in order to assess the prevalence and natural history of food allergies in infancy. The PIFA study was funded by the UK Food Standards Agency.

The PIFA study had a longitudinal prospective cohort design starting from birth. It received approval from Research and Development departments at the Royal Hampshire County Hospital, Winchester and Southampton General Hospital and ethical approval was been granted by North and Mid Hampshire Local Research Ethics Committee and Southampton and South West Hampshire Local Research Ethics Committee (Appendix 3).

4.1.2 PIFA Study design

Pregnant women booked with the Winchester and Eastleigh midwives were approached to participate in the study during antenatal appointments and antenatal classes. Recruitment was aided by study flyers placed in the women's booking paperwork as well as advertisements sent to general practitioners and placed in the National Childbirth Trust (NCT) newsletter. This was all supported by an active education programme for the local midwives undertaken by the PIFA study midwife.

Potential participants had the study briefly described to them and given the detailed study information sheet. For those interested in taking part in the study, an appointment was made for them to meet with one of the study research fellows when the study was explained further, consent taken and the baseline questionnaires completed which collected information on socio-economic, environmental and family allergy history. At this appointment mothers were also invited to keep food diaries for their infants from birth and instructed on how these should be completed.

Parents were contacted after birth to complete the post-natal information required for the baseline questionnaire. From this time and throughout the first year, food diaries were sent out bimonthly for mothers/carers to complete along with freepost envelopes for their return to the study office. In with this mailing was a symptom sheet to be sent back with the food diaries. These enabled parents/carers to inform the study team prospectively of any symptoms their infant was suffering from that may have been food allergy related. Parents were contacted by a member of the study team if symptoms persisted for two or more months.

When each infant reached the age of 12 and then 24 months they were contacted by a member of the study team to arrange an appointment to administer the telephone questionnaire. This covered aspects of the infants nutrition including breast feeding and the introduction of solids, symptoms suggestive of atopic diseases and their treatment, detail of any allergic reactions to food, the use of antibiotics by the child, their family's health, environmental exposures such as exhaled cigarette smoke, and childcare. The questionnaire took between 30 to 60 minutes to complete; those members of the study team who administered it had been trained to do so.

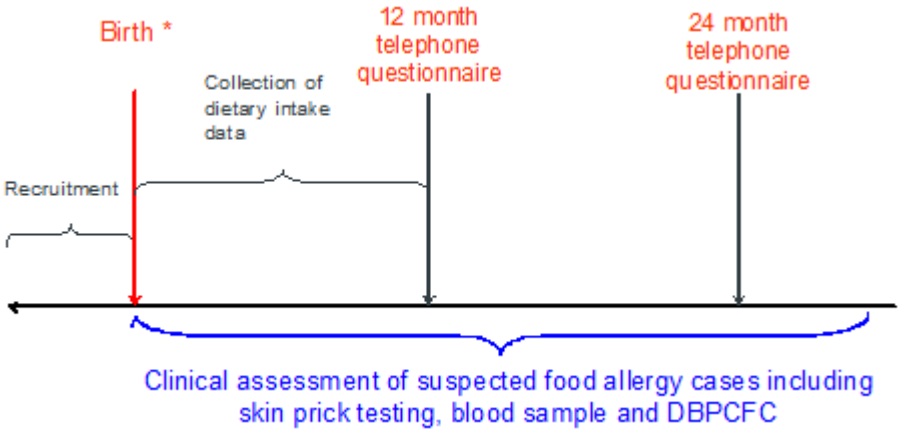

Figure 4.1 Diagrammatic representation of study design of the PIFA Study.
Pregnant women were recruited at mid-trimester and their newborn child was followed until 2 years of age. Prospective food diaries were completed for the first year of life. Routine questionnaire assessment was undertaken at 12 and 24 months. If a participant became symptomatic with possible food allergy, they, and two age matched controls, were assessed.

Infants were not seen for a clinical assessment unless there was a possibility they had undergone an adverse reaction to food. Parents of participants in the birth cohort were asked to contact the study team if they thought their child developed signs of atopic disease. Examples of such symptoms were atopic dermatitis, recurrent wheeze, recurrent vomiting or diarrhoea and adverse reactions to a food. As part of the

information they received with their food diary at 2 monthly intervals, parents/carers were reminded to contact the study office if they suspected their child might have had a reaction to food. In addition, any possible symptomatic children whose parents had not contacted the study team could be identified by their answers to the 12 and 24 month questionnaire.

For every participant with a potential food allergy, two randomly selected age-matched control children from the birth cohort were invited to attend the Wellcome Trust Clinical Research Facility (WTCRF) for an assessment visit. Two controls were selected to increase the statistical power and allow for any drop out of controls during follow up. The controls were selected by approaching parents of existing PIFA study infants with birthdays just before or after the index participant until two controls were found. Potential control participants were invited to attend the WTCRF where they were assessed for any signs of allergic disease using the same criteria used for possible food allergic infants (except for skin prick testing). That is, they underwent assessment for eczema (using the SCORAD system), assessment for any respiratory symptoms and had a blood sample taken for determination of SpIgE to the same panel of allergens used for assessing the symptomatic infants. However, they were not skin prick tested. In addition, the EuroPrevall symptomatic questionnaire was completed and a physical examination by the attending pediatrician was also completed as per the EuroPrevall study protocol. If there was no indication that the infant had any food allergy, and if the SpIgE to any food was less than 0.35kUl/L, then the infant was selected as a control.

4.1.3 Food Allergy diagnosis

Possible cases were triaged via telephone and those fulfilling the EuroPrevall-wide criteria for assessment were invited to the WTCRF for a symptomatic visit. The visit consisted of skin prick testing, blood sample, eczema assessment (using the SCORAD system), and assessment for any respiratory symptoms. The EuroPrevall symptomatic questionnaire was completed and a physical examination undertaken by the attending paediatrician who followed the EuroPrevall protocol for the examination.

Any infant with serum specific IgE (SpIgE) to food ≥0.35kUl/L, and/or a positive skin prick test and/or a convincing clinical history of food allergy was placed on an exclusion diet for the suspected food. If symptoms improved then the child attended the WTCRF for a DBPCFC. The final outcome measure for this study was food allergy diagnosed by DBPCFC.

For details of KG's involvement in the PIFA study see Appendix 4.

4.2 Thesis study design

The study design is a 'nested within a cohort, case control' study with the subjects being infants diagnosed as having a food allergy by DBPCFC by the age of 2 and their two age-matched controls.

Food diaries for infants diagnosed with a food allergy (n=41)) and 2 age-matched controls (n=82) were analysed. Additional data relating to these infants was obtained from the PIFA study databases and this included demographic background data for the infants, maternal diet and health data, birth data, additional dietary intake data collected as part of the 12 and 24 month questionnaires and detailed allergy outcome data.

All data except that for of the food diary data was in database form, but the food diary data required processing and analysing to convert to a format that could be used to analyse with the other variables.

4.2.1 Dietary Assessment Methodology

Both maternal dietary intake data during pregnancy and infant dietary intake data for the first year of life were collected.

4.2.1.1 Maternal dietary intake information

Maternal dietary intake information during pregnancy was collected as part of the baseline questionnaire completed either in the last two weeks of pregnancy or the first few weeks after delivery, depending on when the mother was recruited into the PIFA study. If mothers were recruited before the last two weeks of pregnancy, they were asked to complete the maternal dietary intake questions in the first few weeks after the birth since maternal dietary intake can change in the last few weeks of pregnancy.

The maternal diet questions took the form of asking the mother whether they ate a particular food/food group. If the response was 'yes', they were asked the subsequent question as to whether they had eaten 'more, less or the same amount' of that food during pregnancy compared to when they were not pregnant. If the mother reported that they did not eat the food, they were asked whether they never ate it or whether they ate it before becoming pregnant but now avoided it in their diet. The foods/food groups detailed in the questionnaire were: milk and other dairy products; soy and soy products; eggs and foods containing eggs; peanuts and foods containing peanuts; tree nuts and food containing tree-nuts; seeds and foods containing seeds; fish and fish products; shellfish and foods containing shellfish; cereals and cereal products;

vegetables; fruit; and meat and meat products. A copy of the questions can be found in Appendix 5.

The same questions were asked to determine maternal dietary intake during lactation.

The answers to the questions concerning these 13 foods/food groups were combined and recoded to give a categorical variable of 'Was any food avoided during pregnancy/lactation'.

4.2.1.2 Infant dietary intake information

At recruitment into the birth cohort study, all parents were invited to complete diaries about their children's dietary intake from birth until their child's first birthday. Since the primary purpose of PIFA was a food allergy prevalence study, if parents did not wish to complete the food diaries but still wanted to take part in the study this was allowed; we did not wish food diary completion to adversely affect recruitment onto the prevalence study. Food diaries consisted of a front sheet detailing how to complete the diary and four A4 sheets each with a table to record the child's intake over a week (Appendix 6). Parents/carers were asked to record anything their child ate or drank. They were asked to note the name and manufacturer of any commercially prepared food consumed by their child. They were also asked to record details of any infant formula given. If homemade food was given, parents/carers were asked to give details of ingredients including any recipes used. Parents/carers were given these instructions at recruitment along with the first diary set so they could start recording their infant's intake immediately after birth. Further diary sets were sent out every two months with parents being asked to return each diary to the study office once it was completed and freepost envelopes were provided for this purpose.
It was recognised that food diary completion is a difficult task, so to reduce the work involved in completing the food diary records it was decided that quantitative data would only be recorded once in a four week period; for the other weeks, parents/carers were requested to just record what the child consumed (i.e. they did not need to record details of amounts of food taken). Once the diaries were received into the study office they were studied to ensure they were fit for purpose. If they lacked adequate detail (e.g. type of infant formula given) the parents were contacted by phone so this data could be recorded.

4.2.1.2.1 Dietary intake data processing

The diet diary information was processed in two different ways to facilitate analysis. The first was a unique form of analysis designed by the researcher (KG) which focused on capturing the pattern of the whole diet from week to week (described in section 4.2.1.2.1.1) and the second was a more traditional analysis which used the

recorded quantitative data to provide a nutrient profile of the infant's dietary intake (described in section 4.2.1.2.1.2).

4.2.1.2.1.1 Food diary processing to capture dietary patterns

The data from each infant's weekly diary was used to capture dietary pattern information for that infant ensuring all available information about an infant's diet was used in the dietary pattern analysis and in the determination of time specific events such as when solids were first introduced into the diet and the age breast feeding stopped. This was achieved by recording the detail of the foods/ingredients the child had eaten each week by using an excel data entry front screen with each week having a new weekly front screen (see Figure 4.2). If a food/ingredient was present in the diet then the relevant box on the weekly excel data entry front screen was selected. Details of ingredients were obtained from the relevant food companies and from the recipe information provided by the parent/carer. If recipe/ingredient information was not provided then ingredients of 'standard' recipes from food composition tables (222) were used. Each infant's resultant excel data file from the weekly excel front screens was run through an SAS data manipulation programme (written for the purpose) which converted the weekly 'yes/no' data of what foods and ingredients were present in the infants diet into new variables such as: the number of weeks an infant was breastfed; when an infant first had a particular food ingredient; how many weeks in total an infant ate a food; and how many consecutive weeks they had a particular ingredient. These new SAS variables were then imported into SPSS where further analyses could be run including determination of what type of solid food was first introduced into the diet, and the number of weeks the infant was concurrently fed solids and breast milk. The excel data entry sheet and the SAS data manipulation programme were written by a data manager (Joe Maskell) under direction from KG.

The data obtained by this methodology provided information on every week an infant had a food diary completed and was used in work for objectives 4 and 5[Footnote]. Since all available data was used in these analyses, any observed effects may be subject to 'reverse causality' (which is where behaviour may inaccurately be seen to be a cause of symptoms but it is actually an effect of symptoms) and therefore the results of analyses carried out on this data need to be interpreted with care.

Objective 4: Use of prospective food diary data to determine the nature of the weaning diet for children diagnosed with food allergy by the age of 2 years and their two-age matched controls.
Objective 5: Use of prospective food diary data to identify dietary patterns in children diagnosed with food allergy by the age of 2 years and their two-age matched controls.

There were two types of missing dietary intake data, either the occasional day or sometimes a whole week. The latter case could occur, for example, if the family was away on holiday. For this dietary pattern work, the missing data was dealt with in the following different ways:

1. If the diary records showed the infant was breastfed before and after the missing data period it was assumed the infant was also breastfed during the missing data period and this information was entered into the excel spreadsheet.

2. If any new food (including infant formula) was present in the diet after a period of missing data, the mother was contacted to ask when the food was first introduced and how often the infant received that food. This information was then entered into the excel spreadsheet.

3. For foods that were routinely included in the diet before a period of missing diet data and which were also present after the missing data period, they were entered into the excel spreadsheet for the missing period. This was usually the case for foods such as potatoes, carrots, apples, yogurt/fromage frais.

4. For foods that were only included in the diet infrequently (ie less than once a week) the data was marked as 'missing'.

This method of processing meant that values for foods which formed a regular (more than once a week) part of the infant diet could be imputed for the dietary pattern analysis, thus minimizing the effect of missing data on recognition of the infant's routine intake. However, intake of infrequently eaten items remained unknown for the weeks in which there was missing data.

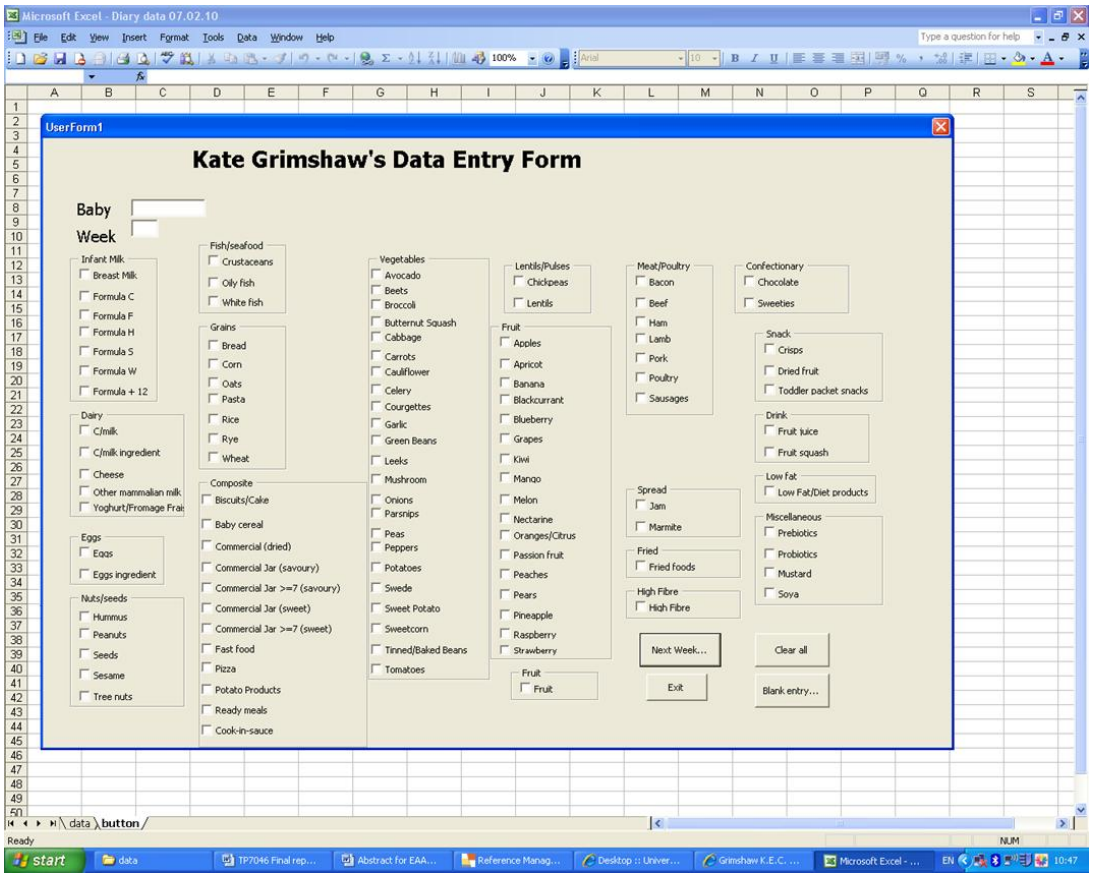

Figure 4.2 Screenshot of Excel data entry front sheet used to record what foods were eaten by the infant that week. *This figure shows the excel data entry screen. The tick box was selected if the infant had eaten the food that week this then populated the excel data sheet with either a 1 if the food had been eaten that week or a 0 if it had not eaten that food.*

4.2.1.2.1.2 Food diary processing for nutrient analysis.

The fourth week of every four week food diary contained quantitative detail of the usual intake data. In addition to being used in the analysis into the patterns of infant dietary intake (see section 4.2.1.2.1.1), these quantitative food diaries were analysed to determine the mean daily nutrient intake data for infants diagnosed with a food allergy and their two age-matched controls (objective 3). Analysis used the dietary analysis package 'CompEatPro' [Nutrition Systems] that had been modified to include nutritional data for all formula milks and baby food products recorded in the food diaries. This information was obtained from the relevant food companies and was current for the time the food diaries were completed. Weights for the portion sizes recorded were established by weighing the household measures recorded by mothers. Homemade mixed purees were assumed to consist of equal proportions of the ingredients recorded and whilst this may not have been 100% accurate in all cases, it ensured continuity of entry for all diaries. If any quantitative diet diary data was

missing or was not completed in sufficient detail then the infants' intake for that month was considered missing.

Many infants received breast milk and 'CompEatPro' had nutritional values for breast milk within its database. Unfortunately, since many mothers did not record the duration and frequency of breast feeds, it was not possible to use an algorithm derived from published intake data to estimate breast milk intake (207;223). However, a guide giving average breast milk intake by age (224) allowed an estimated value for breast milk intake to be entered into the database. The quoted average intake by age was quoted as follows:

0-2 months- 2-5oz per feed- 26oz per day (737g)
2-4 months- 4-6oz per feed- 30oz per day (850g)
4-6 months- 5-7 oz per feed- 31 oz per day (878g)
(1 oz = 28.35g)

These values were obtained from data from a number of research studies and agree with values obtained from other literature (225-227). Although these values are estimates and cannot take into account the differences there are between each mother's milk, using them in all cases does ensure each infants analysis has been completed in the same manner. Additionally, since mothers in this study recorded the number of breast feeds given in a day, the volume per feed was used in the analysis as opposed to the fixed volume per day. This made the analysis more accurate than using a fixed volume per day as not all infants of the same age feed for the same number of times per day.

'CompEatPro' was used to analyse the food diary records that had been entered for all nutrients and mean values for nutrients over 7 days were produced. These values were then transferred to the Statistical Package SPSS for further analysis. The data from the diaries was entered into the nutritional analysis programme by KG and two other nutritionists (Erin Oliver and Karen Scally) who worked under the direction of KG. All coding practices were precisely described to minimise coding errors between the three researchers involved in coding the nutrient content of the diaries. This included: standardised portion sizes; standardised recipes when detail of those used were not given by the mother; and standardised conversion factors for converting imperial measurements into metric measurements. To establish the degree of inter-researcher variability in the diary coding, all three workers coded a complete set of diaries for 10 infants independently of each other (these amounted to 130 weeks diaries for each researcher). The mean daily intake and its standard deviation for each nutrient of interest was determined using each researchers results for each of the 13

time points to give an indication of the variability within the analyses for each time point. This is shown in Appendix 7. For 92 of the 117 variables the standard deviation was less than 15% of the mean showing variance between the values determined by each different researcher for each nutrient across the 10 infant's diary was acceptably small. No standard deviation was above 20% of its mean for any nutrient at any time point.

All processing and analyses of the quantitative nutritional data was done by KG.

The data obtained by this methodology was used in work for objective 3 [Footnote]. Since only the macro and micro nutrient intake prior to diagnosis was of interest to address this objective, only the quantitative diet data of symptomatic infants prior to their symptoms starting was used. Additionally, the dietary intake data from each age-matched control was only analysed to the same time point as their matched symptomatic infant. This is different from the analyses looking at the infant dietary patterns which used all available dietary data for the symptomatic infants and their age matched controls.

4.3 Sample size and statistical power

The PIFA study was part of a trans-European birth cohort study investigating food allergy prevalence (EuroPrevall) and both its study objectives and proposed sample size were designed to meet the requirements of the larger birth cohort study. Whilst it was possible to make minor changes to the protocols of the PIFA and Thesis studies, major protocol changes that would impact on the ability of the study to meet the EuroPrevall objectives were not possible. As a consequence, the thesis study may not have been adequately powered to answer the proposed study hypotheses. To ascertain whether this was the case, power calculations were carried out for all thesis study hypotheses. For hypothesis 1 and 2, results from the thesis study were used to carry out *post hoc* analyses to give an indication of what numbers would have been required to adequately answer the hypotheses. For hypothesis 3 and 4, the power was calculated from the proposed effect size to be detected for 90% power at 5% level (0.5 standard deviation) from power tables obtained from Cohen, 1988 (228). Details of the numbers required for each hypothesis are shown in Table 4.1

Objective 3: Determination of mean daily nutrient intake data from quantitative prospective food diary data for infants diagnosed with food allergy and their two-age matched controls.

Table 4.1 Results of power calculations on the study hypotheses to indicate the number of infants required to obtain a statistically significant result at α level of 0.05 and a power level of 90%

Hypothesis	Number of symptomatic infants	Number of control infants	Total number of infants
1a. Children who develop food allergy by two years of age will have a 5% lower rate for the initiation of breast feeding than children who do not develop a food allergy	1049	2098	2202
1b. Mean breastfeeding duration for children who develop food allergy by two years of age will be 4 weeks less than for children who do not develop a food allergy	341	682	1023
1c. Mean exclusive breastfeeding duration for children who develop food allergy by two years will be 4 weeks less than for children who do not develop a food allergy	65	130	195
1d. Mean duration of concurrent breastfeeding with any cows' milk protein for children who develop food allergy by two years will be 4 weeks less than for children who do not develop a food allergy	185	370	555
2. Children who develop food allergy by two years of age will have solid foods introduced into their diet a mean of 4 weeks earlier than children who do not develop a food allergy	21	42	63
3. Children who develop food allergy by two years of age will have a 0.5 standard deviation difference between the mean intake of energy, protein, fat, iron, zinc, selenium, vitamin A, vitamin C and vitamin E than children who do not develop a food allergy	160	320	480
4. Children who develop food allergy by two years of age will have a 0.5 standard deviation difference in their PCA pattern score than children who do not develop a food allergy	160	320	480

The results from the power calculations for hypothesis 1, 3 and 4, demonstrate that the thesis study is underpowered for these statistical tests. This means there are insufficient numbers in the groups to be able to detect small variable differences which may be important in the development of food allergy. Consequently, variables with values approaching significance also need to be considered in the overall interpretation of the study results. For hypothesis 2, if the difference between the groups is 4 weeks or more then the study is adequately powered. However, if the difference between the groups was not so large then more infants would be needed in the study groups. For example, for a difference of 3 weeks, the numbers would need to be 39 and 78 and for a 2 week difference the numbers would need to be 85 and 170.

In addition to considering that the null hypothesis may be accepted incorrectly due to insufficient numbers, in this study there is also a possibility of rejecting the null hypothesis incorrectly since a number of the analyses (particularly those involving background characteristics and nutrient content of the diet) involve running multiple comparisons on the data. This increases the likelihood of an analysis showing an association between one of the variables and allergy outcome to be below the 5% level. This leads to a situation where the observed association is more likely to be due to chance than the results of the statistical test implies (known as a type 1 error).

Overall, due to the insufficient powering of this thesis study and the multiple tests that have been carried out for some variables, the results needed to be interpreted with care and considered along with the clinical relevance of any observed difference between the groups. For example, for a variable which is significantly different (or is approaching being significantly different), is the observed variable difference seen in the study population feasible to achieve in the general population, and if so, would the observed results in allergy outcome be worth the effort to achieve such a change?

4.4 Statistical analyses

A descriptive analysis of the baseline characteristics of the infants involved in the study (n=123) was carried out using SPSS version 17. Continuous variables were described in terms of means and standard deviation or medians and ranges depending on their distribution. Categorical variables were described in terms of numbers and percentages.

Descriptive statistics were used for the investigation into the relationship between breast feeding and cumulative incidence of infant food allergy (Hypothesis 1) on all available dietary intake data. Chi-square analysis for categorical variables and

Mann Whitney U test for continuous variables (since they were not normally distributed) were then carried out.
For investigating the effect of the timing of infant formula and solid introduction and allergy outcome (Hypothesis 2), descriptive statistics, chi square and Manny-Whitney U test were used on all available dietary intake data. Kaplan Meier survival analysis was also used as an additional analysis to establish more detail about the timing of solid introduction into the infant's diet.

For assessing the effect of the macro and micro-nutrient content of an infant's diet on food allergy development (Hypothesis 3), a mixed design RM-ANOVA was used looking at dietary intake data prior to diagnosis for the symptomatic infants and the same time point for their corresponding age-matched control. Two measures from the RM-ANOVA were considered; the ''within subject' factor and the 'between subject' factor. The 'within subject' factor looked at whether the effect of time affected the intake of the macro/micronutrient differently between the two groups. The 'between subject' factor looked at whether intake was significantly different between the two groups over the whole experimental time period. A one-way Analysis of Variance (ANOVA) was also used to establish the mean values with their 95% Confidence Intervals for Energy, Protein, Fat, Iron, Zinc, Selenium, Vitamin A, Vitamin C and Vitamin E.

To determine whether an infant's specific feeding pattern affected food allergy development (Hypothesis 4), principal component analysis was used to establish patterns within the infant's diets followed by a Mann Whitney U analysis was used to establish whether there was a difference in mean scores for these patterns between symptomatic and control infants.

Finally, a binary logistic analysis was carried out to further investigate the variables which the previous analyses had found to be associated with the cumulative incidence of food allergy at the age of two years.

5. RESULTS

5.1 Baseline characteristics of mothers and infants included in study

Characteristics of the women and infants included in the parent PIFA study (n=1170) and this thesis study (n=123) are detailed in Table 5.1. There was no statistical difference between the PIFA study infants included in the thesis study compared to those PIFA study infants not included in the thesis study except for the area in which they lived (23.1% of infants not included in the thesis study lived in an urban area compared to 13.8 % of infants included in the thesis study p=0.034) and whether the infant's mother took vitamin D supplements when pregnant (1.4% of mothers of infants not included in the thesis study took vitamin D supplements compared to 2.7% of infants included in the thesis study p=0.023) *data not shown*.

The infants involved the thesis study (which investigated the relationship between infant feeding in the first year of life and subsequent allergy development) were those infants from the main parent (PIFA) study who had been diagnosed as having a food allergy by DBPCFC and their two control infants. For the thesis study cohort (n=123), median maternal age was 33 years (range 19-43) and median infant weight 3420g (range 2160-5060). Infants were born between January 2006 and October 2007. There was no significant difference between infants who developed a food allergy and those who did not for any baseline characteristics (including maternal dietary supplement use) apart from whether the mother was eating a full diet (p=0.032). However, the lack of statistical significance may be due to the relatively small sample size of the thesis study resulting in a lack of power to detect small differences between the two study groups. In particular, this may be the case for maternal asthma (p=0.067) and duration of pregnancy (p=0.062) since these values are approaching statistical significance. Appendix 8 details power calculations for these variables. In addition to the possibility of performing a type 2 error, due to the number of tests carried out, there is be a potential for a type 1 error in these analyses (see section 4.3).

The analysis into background characteristics of the study population suggests a 'full' maternal diet may be protective against the development of allergy. Whether the diet was 'full' or not related to data detailing which, if any, of 13 foods/food groups were avoided. These foods were milk and other dairy products, soy and soy products, eggs and foods containing eggs, peanuts and foods containing peanuts, tree nuts and

food containing tree-nuts, seeds and foods containing seeds, fish and fish products, shellfish and foods containing shellfish, cereals and cereal products, vegetables, fruit and meat and meat products. The responses concerning these foods were combined and recoded to give a categorical variable of 'Was any food avoided during pregnancy/lactation'. 21% of women ate a full diet when pregnant but when the analysis is split by study group, only 9.8% of mothers of symptomatic infants ate a full diet during pregnancy compared to 26.8% of mothers of control infants and this difference was significant ($p=0.032$). The foods most commonly avoided for both groups were peanuts, tree nuts and shellfish. Other commonly avoided foods were eggs, seeds and legumes.

Table 5.1 Characteristics of the mother and infant pairs included in the PIFA study (n=1170) and the thesis study (split according to study group) (n=123)

		All PIFA study infants (n=1170)	Symptomatic (n=41)	Control (n=82)	p value†
Male sex (%)		596 (51.0)	24 (58.5)	43 (52.4)	0.522
Median birth weight (g) [Range]		3465 [1745-5060]	3480.0 [2160-4120]	3370.0 [2270-5060]	0.913§
Median length (cm) [range]		53.0 [41-61]	53.0 [48-59]	52.0 [47-61]	0.909§
Duration of pregnancy		40.0 [34-43]	39.5 [36-42]	40.0 [36-42]	0.062§
Median maternal age (yr) [Range]		32.0 [16-47]	31.0 [19-43]	33.0 [22-42]	0.192§
Median paternal age(yr) [Range]		34.0 [17-56]	33.5 [21-42]	34.0 [23-49]	0.247§
Maternal education (%)	Did not complete basic education	10 (0.9)	0	0	0.448
	Completed basic education	123 (10.5)	4 (9.7)	6 (7.3)	
	Junior college/vocational training	351 (30.0	11 (26.8)	15 (18.3)	
	University/college	681 (58.2)	26 (63.4)	61 (74.4)	
Maternal antibiotic use (%)	During pregnancy	181 (15.5)	9 (22.0)	19 (23.2)	0.379
	During delivery	109 (9.3)	7 (17.1)	8 (9.8)	0.321
	After delivery	153 (13.1)	9 (22.0)	11 (13.4)	0.160
	Whilst breastfeeding	362 (31.0)	16 (39.0)	18 (22.0)	0.349
Maternal Multivitamin use (%)	During pregnancy	504 (43.1)	24 (58.5)	47 (57.3)	0.495
	Whilst breastfeeding	342 (29.2)	9 (22.0)	20 (24.4)	0.404
Maternal Folic	During pregnancy	800 (68.4)	36 (87.8)	69 (84.1)	0.345

		All PIFA study infants (n=1170)	Symptomatic (n=41)	Control (n=82)	p value†
acid supplement use (%)	Whilst breastfeeding	123 (10.5)	7 (17.1)	9 (11.0)	0.320
Maternal Vitamin D supplement use (%)	During pregnancy	12 (1.0)	2 (4.9)	1 (1.2)	0.241
	Whilst breastfeeding	9 (0.8)	2 (4.9)	2 (2.4)	0.444
Maternal Fish oil supplement use (%)	During pregnancy	104 (8.9)	3 (9.8)	13 (17.0)	0.235
	Whilst breastfeeding	69.7 (5.9)	2 (4.9)	5 (6.1)	0.514
Full maternal diet (%)	During pregnancy	55 (4.7)	4 (9.8)	22 (26.8)	0.032
	Whilst breastfeeding	75 (6.4)	6 (14.6)	18 (22.0)	0.114
Maternal Paracetomol use	During pregnancy	791 (67.6)	29 (80.5)	50 (68.4)	0.352
	Whilst breastfeeding	(73.5)	31 (86.1)	47 (70.1)	0.709
Maternal anti-inflammatory use	During pregnancy	66 (5.6)	4 (11.4)	7 (9.5)	0.950
	Whilst breastfeeding	346 (29.6)	16 (44.4)	16 (24.4)	0.017
Maternal antacid use	During pregnancy	383 (32.7)	11 (28.6)	10 (12.2)	0.010
	Whilst breastfeeding	40 (3.4)	0	2 (3)	0.294
Median maternal pre pregnancy BMI [Range]		24.0 [19.5-28.5]	22.9 [16.6-43.0]	22.8 [16.5-49.2]	0.323§
Maternal Asthma (%)		136 (11.6)	11 (26.8)	11(13.4)	0.067
Maternal Allergy (%)		333 (28.5)	22 (53.7)	31 (37.8)	0.105
Maternal smoker (%)	Yes	71 (6.0)	1 (2.4)	3 (3.7)	1.000
Only child (%)		602 (51.5)	24 (58.5)	49 (59.8)	0.570
Urban dwelling (%)		279 (23.8)	8 (19.5)	11 (13.4)	0.378
Animal ownership (%)		569 (48.6)	26 (63.4)	40 (48.8)	0.142

† Chi-square test § Mann-Whitney U test. Significance tests for difference between symptomatic and control infants.

5.2 Characteristics of food allergic infants

Median age for symptoms to start in the food allergic infants was 5.8 months. The most common food reacted to was hen's egg (22 infants) closely followed by cow's milk (20 infants). 25 infants (61%) were sensitised (SpIgE ≥ 0.35kUl/L and/or SPT ≥ 3mm) and, as was the case for foods reacted to, the most common food to which infants were sensitised was egg (18 infants). 12 infants were allergic to more than one food. Infants displayed differing symptoms, and some infants displayed more than one symptom at a time. The commonest symptom was vomiting (17 infants), with eczema being the second most common (12 infants). Six infants displayed symptoms of food allergy whilst receiving only breast milk. Full characteristics of the symptomatic infants can be found in Table 5.2

Table 5.2 Characteristics of infants diagnosed with a food allergy (symptomatic) n=41

Infant	No of diaries completed	Age by which first symptoms had developed (months)	Age at first exclusion (months)	Infant diet at first symptoms	Symptoms	Food allergic to	Sensitisation status
1	10	2	5.5	Breast milk	Eczema	Milk,	Positive (Milk/Peanut)
2	12	0	4	Standard infant formula	Eczema	Milk,	Negative
3	13	0	3.5	Breast milk	Colic/diarrhoea	Milk, Peanut	Positive (Milk/Peanut)
4	12	2	4	Standard infant formula	Colic/diarrhoea	Milk	Negative
5	7	7	6	Breast milk	Vomiting	Soya	Negative
6	7	4	4.5	Standard infant formula and solids	Eczema	Peanut	Positive (Egg/Peanut)
7	13	8	12	Standard infant formula and solids	Urticaria	Bean	Positive (Lentil/Bean)
8	8	12	12.5	Breast milk, standard infant formula and solids	Eczema/diarrhoea	Milk	Negative
9	8	7	7	Breast milk, standard infant formula and solids	Vomiting	Milk	Negative

Infant	No of diaries completed	Age by which first symptoms had developed (months)	Age at first exclusion (months)	Infant diet at first symptoms	Symptoms	Food allergic to	Sensitisation status
10	13	0	3	Standard infant formula	Vomiting	Milk	Negative
11	7	2	3	Standard infant formula	Diarrhoea	Milk, Broccoli, Egg	Positive (Milk)
12	3	1	4	Standard infant formula	Eczema/diarrhoea	Milk, Egg	Positive (Egg)
13	8	7	6	Standard infant formula and solids	Vomiting	Egg	Positive (Egg/Fish)
14	13	8	7.5	Standard infant formula and solids	Vomiting	Egg	Positive (Egg)
15	13	1	3	Breast milk and formula	Diarrhoea/vomiting	Milk, Egg	Negative
16	7	15	15	Standard infant formula and solids	Angio-oedema	Egg	Positive (Egg)
17	8	1	4	Breast milk and standard infant formula	Vomiting	Milk, Egg	Positive (Egg)
18	13	14	4	Standard infant formula and solids	Eczema/vomiting	Egg	Positive (Egg)

Infant	No of diaries completed	Age by which first symptoms had developed (months)	Age at first exclusion (months)	Infant diet at first symptoms	Symptoms	Food allergic to	Sensitisation status
19	13	2	3	Breast milk	Vomiting/diarrhoea	Milk	Negative
20	13	7	7	Breast milk and solids	Eczema/vomiting	Milk, Egg	Positive (Milk)
21	5	7	7	Standard infant formula and solids	Urticaria	Egg, Peanut	Positive (Egg)
22	9	9	8.5	Breast milk and solids	Vomiting	Milk, Wheat	Negative
23	4	12	1.5	Standard infant formula and solids	Urticaria	Peanut	Negative
24	7	7	6.5	Breast milk, standard infant formula and solids	Eczema	Egg	Positive (Egg)
25	13	2	2.5	Breast milk and standard infant formula	Eczema	Milk	Negative
26	13	15	19.5	Standard infant formula and solids	Urticaria	Egg	Positive (Milk/Egg)
27	13	11	6	Breast milk and solids	Urticaria	Egg	Positive (Milk/Egg)

Infant	No of diaries completed	Age by which first symptoms had developed (months)	Age at first exclusion (months)	Infant diet at first symptoms	Symptoms	Food allergic to	Sensitisation status
28	0	10	4	Standard infant formula and solids	Urticaria	Egg	Positive (Egg)
29	13	7	7.5	Breast milk, standard infant formula and solids	Vomiting	Egg	Positive (Egg)
30	4	6	12	Standard infant formula and solids	Flushing	Egg	Positive (Egg)
31	3	9	15	Standard infant formula and solids	Eczema	Peanut	Positive (Peanut)
32	7	2	6	Standard infant formula	Eczema	Egg, Peanut, Wheat	Positive (Egg/Peanut/Wheat)
33	5	6	6	Standard infant formula and solids	None!	Egg, Peanut	Positive (Egg)
34	3	2	3	Breast milk and formula	Vomiting	Milk	Negative
35	11	2	4	Standard infant formula	Vomiting	Milk, Soya	Negative
36	0	10	3	Standard infant formula and solids	Angio-oedema	Egg	Positive (Milk/Egg/Peanut)

Infant	No of diaries completed	Age by which first symptoms had developed (months)	Age at first exclusion (months)	Infant diet at first symptoms	Symptoms	Food allergic to	Sensitisation status
37	8	0	4	Breast milk	Eczema/vomiting	Milk	Negative
38	13	14	2	Standard infant formula and solids	Urticaria	Egg	Positive (Egg)
39	9	1	12	Breast milk	Urticaria	Milk, Egg, Soya, Fish	Positive (Egg)
40	4	4	4	Breast milk and standard infant formula	Vomiting	Egg	Negative
41	8	2	1.5	Breast milk and standard infant formula	Vomiting	Milk	Negative

Positive sensitisation = ≥0.35kUl/L and/or Skin prick test wheal size ≤3mm.

For 9 infants, food diaries were stopped before their symptoms started, but for these infants the required feeding data for all analyses except the nutrient intake calculations (objective 3) was obtained from questionnaire data collected as part of the PIFA study protocol.

5.3 The relationship between breastfeeding (initiation, duration, exclusivity and concurrent feeding) and food allergy development by the age of two years

5.3.1 Initiation, duration and exclusivity of breastfeeding

95% of mothers initiated breastfeeding with the median duration of breastfeeding being 20 weeks (range 0-64 weeks). Median duration for exclusive breastfeeding was 8 weeks (range 1-26 weeks) with 50% of mothers still exclusively breastfeeding at 9 weeks of age. There was no significant difference between breastfeeding initiation, duration or exclusive breastfeeding duration between the symptomatic and control infants (Table 5.3) although these analyses are under-powered (see Appendix 8) and therefore a small causal relationship may not be identified.

Table 5.3 Breastfeeding characteristics for all infants (n=123) split according to study group

	Symptomatic (n=41)	Control (n=82)	p value
Breast feeding initiation (%)	38 (92.7)	79 (96.3)	0.333†
Median breast feeding duration in weeks (range)	14.0 (0-64)	20.0 (0-52)	0.295§
Median exclusive breast feeding duration in weeks (range)	5.0 (1-26)	8.5 (1-24)	0.933§

† Chi-square test § Mann-Whitney U test

Additionally, there was no statistical difference for breastfeeding duration when the analysis was carried out for each separate food allergen (data not shown). However, it is still worth noting that when comparing peanut allergic infants to control infants, median breastfeeding duration for peanut allergic infants was 9 weeks compared to a median duration of 20 weeks for control infants but this difference just failed to reach statistical significance (p=0.05); this lack of statistical significance may be due to the small sample size of the study which is exacerbated in this sub-analysis.

Kaplan Meier analysis indicated how the nature of breastfeeding differed between the two study groups and is indicated in Figure 5.1. At time point 0 (x-axis), 100% of infants who were initially breastfed were receiving breastmilk (y-axis). Despite the plot

showing some apparent differences in how the two groups breastfed their infants, the difference between the groups was not significantly different (p=0.335, Breslow).

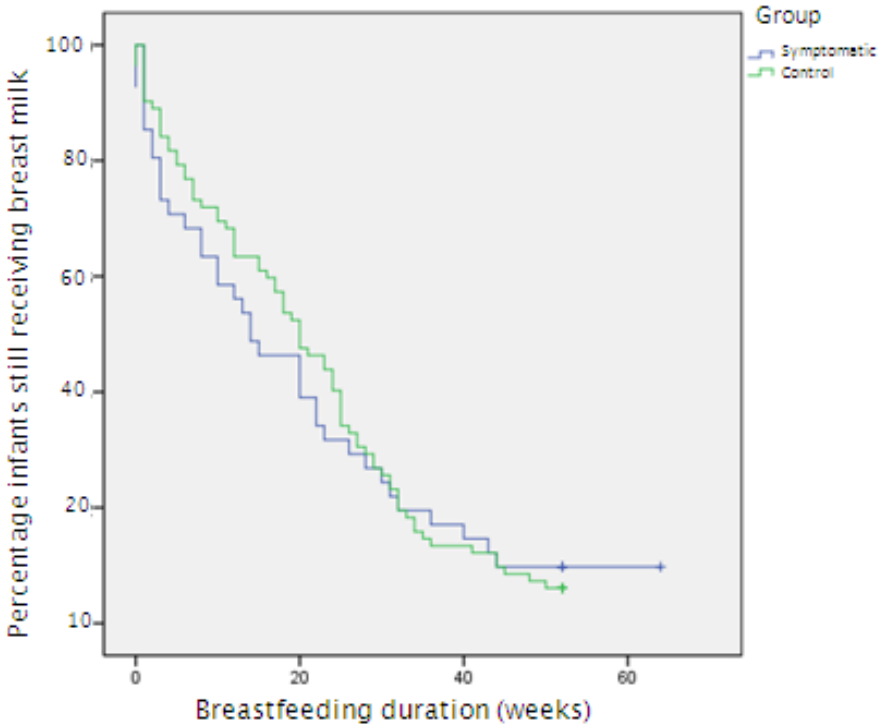

Figure 5.1 Kaplan Meier survival analysis plot showing duration of breastfeeding for symptomatic and control infants

5.3.2 Breastfeeding and concurrent complementary feeding

5.3.2.1 All infants, any solids

The mean duration of concurrent breastfeeding and complementary feeding for all infants was 6.0 weeks (median 0; range 0–38; IQR =9). For symptomatic infants it was 5.4 weeks (median 0, range 0–38; IQR 9) and for control infants it was 6.3 (median 0, range 0–33; IQR 9). This difference was not statistically significant (p=0.303) but again this analysis is underpowered (see Appendix 8). Details of the overlap between the introductions of each allergen whilst the infant was still being breastfed are given in Table 5.4. Since no allergenic foods, except for cows' milk, were fed concurrently with breast milk in enough infants to provide a valid statistical result, the analysis was only run for concurrent breastfeeding and cows' milk in any form. There was a statistically significant difference between the two study groups for the duration of concurrent breastfeeding and feeding with cow's milk in any form and the duration of

concurrent breastfeeding and formula feeding (p=0.047 in each case). To investigate whether the duration of overlap was important the variables were recoded to give 'concurrent breastfeeding' yes/no for each food. Again there was a difference between the groups seen for concurrent feeding with cow's milk in any form and concurrent feeding with infant formula (p=0.015 and p=0.022 respectively) suggesting that the duration of concurrent feeding was not crucial. Additionally, it may be possible there is a particular age that is important in the relationship, but there was no significant difference between the two experimental groups as to when the overlap occurred (Mann Whitney U p=0.300).

Table 5.4 Characteristics of concurrent breast feeding habits for all infants (n=123) and split according to study group.

Median duration in weeks of concurrent breastfeeding with…	All infants (n=123)	Symptomatic (n=41)	Control (n=82)	p value§
Any solid food (range)	0.0 (0–38)	0.0 (0– 38))	0.0 (0–33)	0.303
Cows' milk – all forms (range)	9.0 (0–23) [N=100 (81%)]	11.0 (0–51) [N= 28 (68%)]	13.0 (0–45) [N=72 (88%)]	0.047
Infant formula (range)	9.0 (0–33) [N=98 (80%)]	15.0 (1–51) [N=28 (68%)]	11.5 (0–45) [N=70 (85%)]	0.047
Whole Cows' milk (range)	0 (0–24) [N=9 (7%)]	No infants concurrently fed [N=0 (0%)]	0 (0–24) [N=9 (11%)]	n/a
Hen's egg – all forms (range)	0 (0–6) [N=17 (14%)]	Insufficient numbers concurrently fed for analysis [N=4 (9.7%)]	0 (0–27) [N=13 (16%)]	n/a
Hen's egg – ingredient (range)	0 (0–6) [N=13 (11%)]	Insufficient numbers concurrently fed for analysis [N=3 (7%)]	0 (0–27) [N=10 (12%)]	n/a
Hen's egg – (range)	0 (0–5) [N=12 (10%)]	Insufficient numbers concurrently fed for analysis [N=2 (5%)]	0 (0–15) [N=10 (12%)]	n/a
Wheat (range)	0 (0–13) [N= 40 (33%)]	0 (0–4) [N=11 (27%)]	1.0 (0–13) [N=29 (35%)]	n/a
Soya (range)	0 (0–16) [N=11 (9%)]	0.5 (0–16) [N=7 (17%)]	0 (0–11) [N=4 (5%)]	n/a
Fish – all types (range)	0 (0–11) [N=27 (22%)]	0 (0–11) [N=8 (20%)]	0 (0–7) [N=19 (23%)]	n/a
White fish (range)	0 (0–25)	0 (0–11)	0 (0–7)	n/a

Median duration in weeks of concurrent breastfeeding with…	All infants (n=123)	Symptomatic (n=41)	Control (n=82)	p value§
	[N=27 (22%)]	[N=8 (20%)]	[N=19 (23%)]	
Oily fish (range)	0 (0-7) [N=15 (12%)]	0 (0-7) [N=4 (10%)]	0 (0-4) [N=11 (13%)]	n/a
Peanut	Insufficient numbers concurrently fed for analysis [N=2 (2%)]	Insufficient numbers concurrently fed for analysis [N=2 (5%)]	No infants concurrently fed [N=0 (0%)]	n/a
Tree nuts	Insufficient numbers concurrently fed for analysis [N=1 (0.8%)]	Insufficient numbers concurrently fed for analysis [N=1 (2%)]	No infants concurrently fed [N=0 (0%)]	n/a

§ Mann-Whitney U test

5.3.2.2 Specific food allergy

As was the case for the timing of solid introduction into the diet, the allergenicity of foods may play a role in the relationship between concurrent breastfeeding and complementary feeding. Therefore, the analysis was further stratified by looking at concurrent breastfeeding with the allergenic food to which the symptomatic infant was reactive. Mean values for duration of concurrent breastfeeding for each symptomatic infant sub-group was compared with the mean duration of concurrent breastfeeding for the control infants (Table 5.5). As for the non-stratified analysis, only the duration of concurrent breastfeeding with cow's milk in any form and infant formula occurred frequently enough to allow statistical analysis, which found a significant difference between the two experimental groups.

VB

Table 5.5 Characteristics of concurrent breast feeding habits stratified by the food each symptomatic infant was allergic to.

Median duration in weeks of concurrent breastfeeding with…	Symptomatic (n=41)	Control (n=82)	p value§
Any food allergic infant (n=41)			
Of any allergenic food (range)	7 (0-51)	9 (0.33)	0.141
Milk allergic infants			
Infant formula (n=15)	4 (0-32)	11.5 (0-45) n=70	0.017
Whole Cows' milk (n=0)	No infants concurrently fed	0 (0-24) n=9	n/a
Cows' milk (all forms) (n=15)	7 (0-35)	13.0 (0-45) n=72	n/a
Egg allergic infants (n=1)			
Hen's egg (n=0)	No infants concurrently fed	0 (0-15) n=10	n/a
Hen's egg (ingredient) (n=1)	0 (0-32)	0 (0-27) n=10	n/a
Hen's egg (all forms) (n=1)	0 (0-32)	0 (0-27) n=13	n/a
Wheat allergic infants (n=1)			
Wheat	4.5 (0-9)	1.0 (0-13) n=29	n/a
Soya allergic infants (n=1)			
Soya	0 (0-36)	0 (0-11) n=4	n/a
Fish allergic infants (n=0)			
White fish	No infants concurrently fed	0 (0-7) n=19	n/a
Oily fish	No infants concurrently fed	0 (0-4) n=11	n/a
Fish (all types)	No infants concurrently fed	0 (0-7) n=19	n/a

Median duration in weeks of concurrent breastfeeding with…	Symptomatic (n=41)	Control (n=82)	p value§
Peanut allergic infants (n=0)			
Peanut	No infants concurrently fed	No infants concurrently fed	n/a

§ Mann-Whitney U test

5.4 The relationship between the timing and nature of infant formula and solid food introduction into the infant's diet and food allergy development by the age of two years

5.4.1 Infant formula introduction

Median age at first formula use for all infants was 6 weeks (range 1-34 weeks). Formula was first introduced to the symptomatic infants at a median age of 4 weeks (range 1-29 weeks) and for the control infants at the median age of 6 weeks (range 1-34 weeks). This observed difference between the groups did not meet statistical significance (p=0.478) (Table 5.6).

54% of infants took an infant formula that contained prebiotics. 39% of infants who went on to develop a food allergy used such a formula compared to 61% of infants who did not develop a food allergy. This difference did not reach statistical significance (p=0.094) but that may be due to lack of statistical power due to small study numbers (Appendix 8).

5.4.2 Solid food introduction

5.4.2.1 All infants (n=123), any solids

Mean age of any solid introduction was 20.3 weeks. Figure 5.2 demonstrates that although age of solid introduction appears to be normally distributed there are peaks within the distribution at ages of 17, 21 and 25 weeks of age, so non-parametric tests will be used in the further analyses.

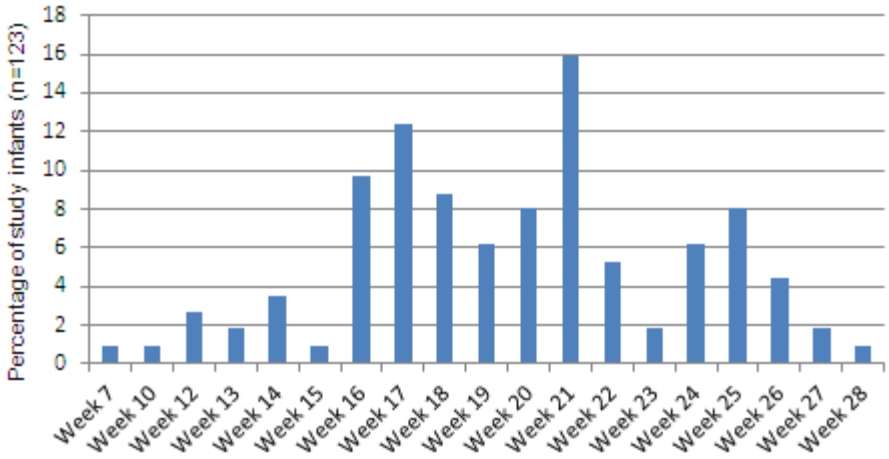

Figure 5.2 Age at solid introduction for all study infants (n=123).

The distribution of the introduction of other allergenic foods was not normal (Kolmogorov-Smirnov $p < 0.05$) so non-parametric tests were used to assess statistical significance of any observed differences between the groups. There was a significant difference in the age solids were introduced between symptomatic and control infants ($p=0.044$) and also for the age cow's milk was introduced as an ingredient ($p=0.049$), the age whole cow's milk was introduced ($p=0.004$) and the age peanut was first introduced ($p=0.037$) (Table 5.4). The age that hen's egg in all forms and as an ingredient was introduced into the infants diet just failed to reach statistical significance ($p=0.087$ and 0.067 respectively) but this may be due to the relatively small sample size resulting in inadequate power in the analysis to detect small differences between the groups.

The age that whole cow's milk was introduced may be different between the groups because cow's milk allergic infants would not have had whole cow's milk introduced into their diet (since infants diagnosed with a milk allergy would not yet have had cows' milk as cows' milk introduced into their diet as it is not recommended until the age of 1 year). This is known as reverse causality. The analysis was re-run excluding the milk allergic infants and the difference between the groups was no longer significant ($p=0.064$). The difference in age peanut is first introduced into the diet is also likely to be due to a response to study procedures. It appears that symptomatic infants had peanuts introduced into their diet sooner than for controls. This is likely to be due to the fact that symptomatic infants had been skin prick tested to a panel of allergens and, since mothers had seen that their infants were not sensitised to peanut, they were more willing to introduce it into their diet. Mothers of control infants did not have this visual cue so were less likely to introduce peanut earlier.

Table 5.6 Age at introduction of complementary foods given for all infants (n=123) and for study group.

Median age in weeks at introduction	All infants (n=123)	Symptomatic (n=41)	Control (n=82)	p value§
Age at solid introduction (range)	20 (7-28)	18 (12-27)	20 (7-28)	0.044
Infant formula (range)	3 (1-40)	3 (1-40)	5 (1-34)	0.478
Median Cows' milk – ingredient (range)	25 (13-52)	22 (13-52)	26 (16-52)	0.049
Cows' milk (range)	46 (23-52)	52 (33-52)	42 (23-52)	0.004
Cows' milk – all forms (range)	4 (1-52)	3 (1-52)	5 (1-52)	0.283
Hen's egg (range)	40 (21-52)	40 (21-52)	39 (26-52)	0.930
Hen's egg – ingredient (range)	38 (17-52)	43 (29-52)	36 (17-52)	0.067
Hen's egg – all forms (range)	35 (17-52)	39 (20-52)	35 (17-52)	0.087
Wheat (range)	27 (13-52)	27 (13-52)	26 (13-50)	0.573
Soya (range)	29 (17-52)	29 (17-52)	30 (19-52)	0.569
White fish (range)	30 (20-52)	31 (20-52)	29 (22-52)	0.980
Oily fish (range)	35 (17-52)	33 (31-52)	36 (17-52)	0.217
Fish – all types (range)	29 (17-52)	29 (20-32)	29 (17-52)	0.860
Peanut (range)	52 (26-52)	52 (26-52)	52 (35-52)	0.037
Tree nut (range)	52 (30-52)	52 (30-52)	52 (40-52)	0.258
Sesame (range)	52 (21-52)	52 (21-52)	52 (31-52)	0.053

§ Mann-Whitney U test

A Kaplan Meier survival analysis showed how infants had solid food introduced into their diet over time (Figure 5.3). At 0 weeks no infants had solids introduced into their diet, represented by 100% on the y axis (100% of infants had not yet received solids into their diet). Figure 5.3 shows graphically that, apart from at the very beginning of the plot, infants who were already food allergic or who were to become so were introduced to solids earlier than the control infants. Statistical analysis of the

difference between the survival curves for each group shows the difference is significant (p=0.036, Breslow test).

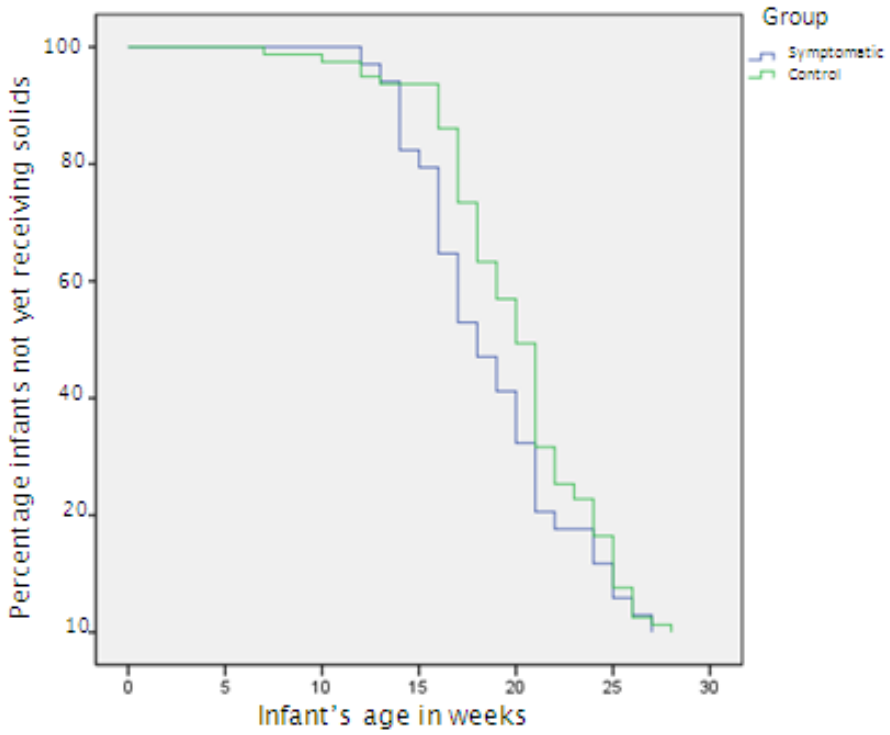

Figure 5.3 Kaplan Meier survival analysis plot showing timing of solid introduction for symptomatic and control infants.

Additionally, the Kaplan Meier survival analysis showed that 54% of symptomatic infants had received solids by 16 weeks with 61% receiving solids by 20 weeks compared to 40% and 52% respectively for control infants. By plotting the percentage of infants who had received solids for each study group separately against time as a *post hoc* analysis, it can be observed that the plots have a similar pattern apart from the period between 12 and 16 weeks (Figure 5.4). During this time 15% of symptomatic infants were introduced to solids but only 9% of control infants were introduced to solids. To investigate this difference further, analyses were carried out which stratified the timing of solid introduction into two groups, solid introduction before and including 16 weeks of age and solid introduction at 17 weeks of age and older. This was done by creating a categorical variable 'introduced to solids at 16 weeks or earlier' and then carrying out a chi-square analysis. 35% of symptomatic infants where introduced to solids at 16 weeks or earlier compared to 14% of control infants and this difference was statistically significant (Chi square p=0.011).

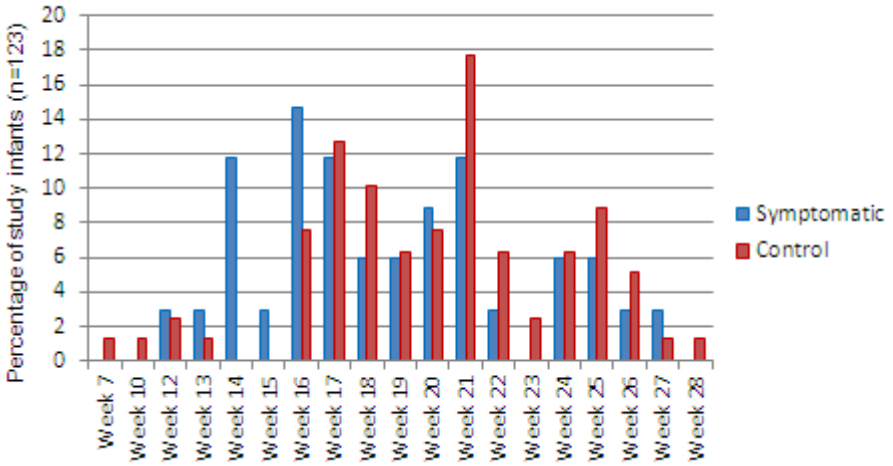

Figure 5.4 Age at solid introduction for all study infants (n=123) split by study group.

To further investigate any possible relationship between solid introduction and allergy development, a *post hoc* Kaplan Meier analysis of the timing of cows' milk as an ingredient was carried out since the Mann Whitney U test had shown the age this occurred was significantly different between the groups (Table 5.6). Results of the Kaplan Meier analysis showed the age by which 25% of infants had received cows' milk as an ingredient was 17 weeks of age for symptomatic infants but 21 weeks of age for control infants. Similarly, the age at which 50% of infants had received cows' milk as an ingredient was 22 weeks of age for symptomatic infants compared to 26 weeks of age for control infants (data not shown) with the Kaplan Meier analysis showing the difference in the survival curves for the two study groups was statistically significant (p=0.033, Breslow test).

As was the case for age at the introduction of solids, the percentage of infants who had received cows' milk as an ingredient for each study group was plotted against time (Figure 5.5). From this plot it can be observed that the percentage of infants receiving cows' milk as an ingredient for the first time between the ages of 19 and 29 weeks were similar between the groups but that they differed up to week 19 and from week 29 onwards. Both, neither, or just one of these time differences may be causal and therefore were investigated further. Up until 19 weeks of age, 33% of symptomatic infants had received cows' milk as an ingredient compared to 8% of control infants. Stratified analyses into introduction of cows' milk as an ingredient before and including 19 weeks of age and introduction from 20 weeks onward was carried out by creating a categorical variable of 'had cows' milk as an ingredient at 17 weeks or younger' and then carrying out a chi-square analysis. The difference between the groups was statistically significant (Chi square p=0.008). Analysis on the other period

of interest where there was a difference in feeding with cows' milk as an ingredient between the groups was carried out ie introduction of cow's milk as an ingredient before and including 28 weeks of age and introduction from 29 weeks onward. As was carried out previously, a categorical variable was created. By 28 weeks of age, 86% of symptomatic infants had had cows' milk as an ingredient introduced into their diet compared to 72% of control infants. However, Chi-squared analysis showed this difference was not statistically significant (p=0.166), showing the time period of interest for the introduction of milk as an ingredient to be up to 19 weeks of age.

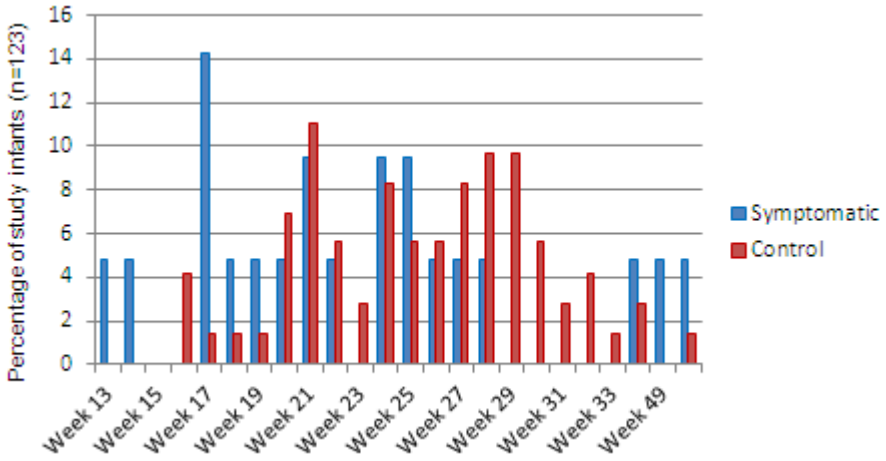

Figure 5.5 Age at introduction of cows' milk as an ingredient for all study infants (n=123) split by study group.

5.4.2.2 Specific food allergy

The allergenicity of foods may play a role in the relationship between the timing of solid food introduction and food allergy development, therefore the timing of allergenic food introduction was compared between the symptomatic and the control infants and then the timing of the introduction of food allergen to which the children subsequently became allergic was investigated. Stratifying the analysis of the relationship between solid introduction and the subsequent development of food allergy in this way did not uncover any statistically significant differences between symptomatic and control infants except for the timing of the introduction of cow's milk as an ingredient (p=0.042) and whole cows' milk (p=0.012) (Table 5.7) but this lack of statistical significance for all allergens except cows' milk may be due to insufficient numbers in the analysis since stratifying the groups further reduced the numbers in the experimental groups.

As was the case when the analysis was carried out on all infants, this observed relationship with introduction of whole cows' milk (as cows' milk) is likely to be due to reverse causality (since infants diagnosed with a milk allergy would not yet have had cows' milk as cows' milk introduced into their diet as it is not recommended until the age of 1 year).

Table 5.7 Age at introduction of complementary foods stratified by the food each symptomatic infant was allergic to.

Age at introduction (weeks)	Symptomatic (n=41)	Control (n=82)	p value§
Any food allergic infant (n=41)			
Of any allergenic food (range)	3.0 (1–28)	5.0 (1–29)	0.665
Milk allergic infants (n=20)			
Infant formula	5.0 (1–29)	6.0 (1–34)	0.792
Median Cows' milk (ingredient)	24.0 (21–52)	27.0 (16–52)	0.042
Cows' milk	52.0 (52)	43.5 (23–52)	0.012
Cows' milk (all forms)	5.0 (1–21)	6.0 (1–32)	0.456
Egg allergic infants (n=22)			
Hen's egg	50.0 (27–52)	40.0 (26–52)	0.925
Hen's egg (ingredient)	45.5 (30–52)	37.0 (21–52)	0.152
Hen's egg (all forms)	45.5 (27–52)	35.0 (21–52)	0.250
Wheat allergic infants (n=2)			
Wheat	26.5 (26–27)	26.0 (13–50)	0.952
Soya allergic infants (n=3)			
Soya	32.0 (28–35)	29.5 (19–52)	0.865
Fish allergic infants (n=1)			
White fish	33.0 (33.0)	29.5 (20–52)	0.648
Oily fish	0	36.0 (17–52)	utc
Fish (all types)	33.0 (33.0)	29.0 (17–52)	0.499
Peanut allergic infants (n=7)			
Peanut	52.0 (52)	52.0 (35–52)	0.590

§ Mann-Whitney U test; utc =unable to calculate

5.5 The relationship between the macro and micro-nutrient content of an infant's diet on food allergy development by the age of two years

A total of 31 of the food allergic infants had complete food diaries containing data of a good enough quality to allow quantitative data dietary analysis from birth until symptoms developed and these diaries were analysed along with diaries from 62 age-matched controls. Table 5.8 details the baseline characteristics of symptomatic infants included (n=31) and not included (n=10) in this nutrient analysis. The symptomatic infants not included in the nutrient analysis did not differ significantly from the symptomatic infants included in the analysis, although the difference in maternal education and pet ownership between the groups approached statistical significance. Had the sample size been larger this difference may have been statistically significant.

Table 5.8 Characteristics of symptomatic infants included in the nutrient analysis and symptomatic infants who were not included in the nutrient analysis.

		Symptomatic infants included in the nutrient analysis (n=31)	Symptomatic infants not included in the nutrient analysis (n=10)	p value†
Male sex (%)		19 (61)	5 (50)	0.714
Median birth weight (g) [Range]		3590 [2160-4120]	3425 [2750-3830]	0.303§
Median length (cm) [range]		53 [48-59]	53 [46-57]	0.701§
Median duration of pregnancy (weeks) [Range]		39 [35-42]	40 [37-41]	0.220§
Median maternal age (yr) [Range]		32 [19-43]	30 [24-40]	0.110§
Median paternal age(yr) [Range]		34 [21-42]	33.5 [27-41]	0.867§

		Symptomatic infants included in the nutrient analysis (n=31)	Symptomatic infants not included in the nutrient analysis (n=10)	p value†
Maternal education	Completed basic education (%)	3 (10)	1 (10)	0.075
	Junior college/vocational training (%)	8 (26)	3 (30)	
	University/college (%)	20 (65)	6 (60)	
Maternal antibiotic use (%)	During pregnancy	8 (26)	1 (10)	0.758
	During delivery	6 (19)	1 (10)	0.415
	After delivery	6 (19)	3 (30)	0.450
	Whilst breastfeeding	13 (42)	3 (30)	0.201
Maternal Multivitamin use (%)	During pregnancy	20 (65)	4 (40)	0.553
	Whilst breastfeeding	8 (26)	1 (10)	0.710
Maternal Folic acid supplement use (%)	During pregnancy	28 (90)	8 (80)	0.389
	Whilst breastfeeding	7 (23)	0 (0)	0.350
Maternal Vitamin D supplement use (%)	During pregnancy	1 (3)	1 (10)	0.156
	Whilst breastfeeding	2 (6)	0 (0)	0.635
Maternal Fish oil supplement use (%)	During pregnancy	3 (10)	1 (10)	0.733
	Whilst breastfeeding	2 (6)	0 (0)	0.635
Full maternal diet (%)	During pregnancy	4 (13)	0 (0)	0.360
	Whilst breastfeeding	5 (16)	1 (10)	0.656
Median maternal pre pregnancy BMI [Range]		24.4 [16.7-34.2]	24.5 [20.7-43.0]	0.381§

		Symptomatic infants included in the nutrient analysis (n=31)	Symptomatic infants not included in the nutrient analysis (n=10)	p value†
Maternal Asthma (%)		7 (23)	4 (40)	0.413
Maternal Allergy (%)		17 (55)	5 (50)	0.537
Maternal smoker (%)	Yes	0 (0)	1 (10)	0.244
Only child (%)		12 (39)	5 (50)	0.717
Urban dwelling (%)		6 (19)	2 (20)	0.642
Animal ownership (%)		22 (71)	4 (40)	0.084

† Chi-square test § Mann-Whitney U test

Analysis for the nutrient content of the infants' diet was carried out to enable a comparison between the nutrient intake of infants prior to their symptoms of food allergy starting and the nutrient intake of their age matched controls to the same age (objective 3). There were 41 infants diagnosed with food allergy, but ten of these infants were not included in this analysis. This was due to six of them having symptoms which started prior to when their first quantitative diary was kept (week4) and four who did not have diary data returned for week 4 (or beyond) and so could not be included in the analysis. Consequently, the starting number for these analyses was 31, with the same 31 infants included until they became symptomatic or no longer had food diaries returned.

Since the infant's symptoms started at different ages, the number of diaries analysed at each time point decreased (as the infants developed their symptoms). Additionally, some infants who were to go on to be diagnosed as having a food allergy did not have food diaries returned up to the point their symptoms started hence another reason the numbers of dairies analysed reduced between the analysis time points. Table 5.9 details the number of dairies analysed at each time point, how many of these were control infants diaries (always twice the number of symptomatic infant diaries available to be analysed), and how many were the diaries of infants who were to go on to be diagnosed as having a food allergy. Detail of where the 'drop-off' in numbers of diaries occurred and why (whether it was due to the start of symptoms or due to non-return of diary data) is also included in the table.

Table 5.9 Number of diaries analysed for nutrient intake at each time point

	No of infants who developed symptoms	No of infants without diary data	No of symptomatic infants with diary data	No of control infants with diary data	Total no of diaries analysed at time point
Prior to week 4	6	4			
Diary 1 (week 4)			31	62	93
Weeks 5,6, 7	9	4			
Diary 2 (week 8)			18	36	54
Weeks 9,10, 11	0	0			
Diary 3 (week 12)			18	36	54
Weeks 13, 14, 15	2	2			
Diary 4 (week 16)			14	28	42
Weeks 17, 18, 19	0	0			
Diary 5 (week 20)			14	28	42
Weeks, 21, 22, 23	0	0			
Diary 6 (week 24)			14	24	42
Weeks 25, 26, 27	6	0			
Diary 7 (week 28)			8	16	24
Weeks 29, 30, 31	3	1			
Diary 8 (week 32)			4	8	12
Weeks, 33, 34, 35	1	0			
Diary 9 (week 36)			3	6	9
Weeks, 37, 38, 39	0	0			
Diary 10 (40)			3	6	9
Weeks 41, 42, 43	1	0			
Diary 11 (44)			2	4	6
Weeks 45, 46, 47	0	0			
Diary 12 (48)			2	4	6
Weeks 49, 50, 51	0	0			
Diary 13 (52)			2	4	6

Symptomatic infants n=41

The dietary intake data was coded and entered into the dietary analysis package 'CompEatPro' [Nutrition Systems] to give mean energy, protein, fat, iron, zinc, selenium, vitamin A, vitamin C and vitamin E intakes for the infants who were to become food allergic and their age-matched controls (Tables 5.10, 5.11 and 5.12) . Mean percentage weight increase at 12 months for the infants included in the analysis was 266%. The mean percentage weight increase at 12 months for symptomatic infants was 236% and was 281% for control infants. Mean height increase at 12 months was 24cm for all infants, 22cm for symptomatic infants and 25cm for their age matched controls. Since this is in the expected range for weight and height increase in the first year of life (229), it indicates that the infants had adequate nutritional intake to support normal growth.

A mixed design RM-ANOVA was carried out to determine if the nutrient intake prior to symptoms developing in the symptomatic infants was different from the nutrient intake of their control infants. This procedure allows for analysis of the data from each experimental group across the time points of interest whilst taking into account the possibility for a type 1 error (rejecting the null hypothesis incorrectly) because the measures are from the same subjects. As a whole series of data are required for this analysis, the data from diaries 1 to 6 were included to allow the greatest amount of available data over the most time points to be analysed. The RM-ANOVA gives results for 'between-subject' analysis (which looks at all the data across the time points as a whole and compares between the two experimental groups), and 'within-subject' analysis (which looks to see if the difference between any two time points differs between experimental groups). The mixed design RM-ANOVA found no significant difference between the dietary intake of the food allergic infants and their controls for any of the pre-specified nutrients for either the 'between-subject' analysis or the 'within-subject' analysis.

The one-way analysis of variance (ANOVA) found a significant difference between the two groups for protein intake at month 10 ($p=0.05$) and month 11 ($p=0.03$), and vitamin E at month 9 ($p=0.04$) (Tables 5.10, 5.11 and 5.12). However, since this analysis is underpowered for all nutrients (Appendix 9) and is also prone to a type 1 error due to the multiple tests on the same individuals, accurate interpretation of the data is not possible.

Table 5.10 Mean macronutrient intake per day for dietary intake prior to diagnosis for symptomatic infants and their controls.

		Mean Energy (Kcal)			Mean Protein (g)				Mean Total Fat (g)		
Mon	Symptomatic (95% CI)	Control (95% CI)	EAR (kcal/day)	p	Symptomatic (95% CI)	Control (95% CI)	RNI (g/day)	p	Symptomatic (95% CI)	Control (95% CI)	p
Mon 1	518.4 (497.3–539.4)	512.6 (501.4–523.9)	545 males 515 females	0.60	10.1 (9.5–10.7)	9.9 (9.7–10.2)	12.5	0.55	29.9 (28.9–30.9)	29.7 (28.9–30.4)	0.74
Mon 2	573.5 (547.0–600.1)	587.5 (572.2–602.7)	545 males 515 females	0.32	11.0 (10.5–11.5)	11.6 (11.1–12.1)	12.5	0.12	33.6 (31.9–35.2)	33.7 (32.5–34.8)	0.90
Mon 3	587.6 (550.1–625.1)	608.5 (592.2–624.9)	545 males 515 females	0.22	11.3 (10.6–12.0)	12.1 (11.5–12.7)	12.5	0.12	34.0 (31.7–36.4)	34.6 (33.7–35.6)	0.54
Mon 4	643.1 (608.9–677.3)	675.9 (609.1–742.6)	690 males 645 females	0.50	12.9 (11.9–14.0)	13.8 (12.0–15.6)	12.7	0.51	36.4 (34.5–38.3)	37.9 (34.2–41.6)	0.57
Mon 5	688.6 (600.0–777.2)	646.2 (622.5–670.0)	690 males 645 females	0.21	14.9 (11.8–18.1)	13.2 (12.3–14.0)	12.7	0.15	36.8 (33.3–40.2)	35.2 (33.9–36.6)	0.30
Mon 6	707.4 (630.4–784.4)	708.2 (659.4–757.0)	690 males 645 females	0.98	14.5 (12.6–16.3)	15.1 (13.5–16.6)	12.7	0.63	35.5 (32.4–38.7)	35.6 (33.2–37.9)	0.98
Mon 7	812.1 (716.2–908.0)	739.6 (686.2–793.0)	825 males 765 females	0.13	19.6 (13.7–25.5)	19.8 (16.5–23.1)	13.7	0.93	36.7 (32.1–41.4)	31.6 (26.0–37.1)	0.21
Mon 8	774.9 (658.4–891.4)	788.0 (699.9–876.0)	825 males 765 females	0.83	24.9 (17.9–31.9)	20.5 (14.6–26.4)	13.7	0.29	30.8 (19.2–42.5)	34.1 (30.4–37.8)	0.36

		Mean Energy (Kcal)			Mean Protein (g)				Mean Total Fat (g)		
Mon	Symptomatic (95% CI)	Control (95% CI)	EAR (kcal/day)	p	Symptomatic (95% CI)	Control (95% CI)	RNI (g/day)	p	Symptomatic (95% CI)	Control (95% CI)	p
Mon 9	862.6 (610.6–1114.6)	838.3 (721.2–955.5)	825 males 765 females	0.76	26.9 (20.5–33.2)	23.8 (17.7–29.9)	13.7	0.43	33.9 (2.0–65.7)	36.1 (30.2–42.0)	0.72
Mon 10	1047.1 (180.3–1913.8)	881.0 (734.1–1027.9)	920 males 865 females	0.18	34.1 (15.7–83.8)	25.3 (21.1–29.5)	14.9	0.05	46.2 (–52.2–144.7)	35.2 (27.7–42.8)	0.14
Mon 11	1045.3 (93.8–2184.4)	880.8 (645.0–1116.6)	920 males 865 females	0.26	35.3 (21.0–49.6)	27.3 (22.9–32.2)	14.9	0.03	40.6 (37.9–119.1)	36.4 (22.4–50.3)	0.61
Mon 12	1041.8 (495.1–1588.5)	1154.5 (741.0–1568.0)	920 males 865 females	0.60	33.2 (9.3–57.2)	34.2 (25.8–42.6)	14.9	0.82	38.5 (34.8–42.3)	48.5 (30.1–67.0)	0.31
Mon 13	1083.9 (242.7–1925.1)	1085.0 (684.4–1485.6)	920 males 865 females	0.99	37.6 (13.1–62.0)	36.7 (30.0–43.5)	14.9	0.82	42.3 (18.9–103.4)	45.6 (22.3–68.9)	0.78

Values are mean monthly intakes and 95% confidence intervals (CI) for energy, protein, carbohydrate, and fat for symptomatic and control infants. Estimated Average requirements (EAR) given for energy and Reference Nutrient Intake (RNI) stated where one exists.

Table 5.11 Mean mineral intake per day for dietary intake prior to diagnosis for symptomatic infants and their controls.

	Mean Iron (mg)				Mean Zinc (mg)				Mean Selenium (ug)			
Mon	**Symptomatic (95% CI)**	**Control (95% CI)**	**RNI (mg/ day)**	**p**	**Symptomatic (95% CI)**	**Control (95% CI)**	**RNI (mg/ day)**	**p**	Symptomatic (95% CI)	Control (95% CI)	**RNI (µg/ day)**	**p**
Mon 1	1.4 (0.8–2.1)	1.4 (1.0–1.8)	1.7	0.91	2.3 (1.9–2.7)	2.3 (2.1–2.6)	4.0	0.94	8.3 (7.6–9.1)	8.5 (8.0–9.1)	10	0.70
Mon 2	1.2 (0.6–1.8)	2.0 (1.3–2.7)	1.7	0.15	2.4 (2.1–2.8)	2.9 (2.5–3.3)	4.0	0.09	8.9 (8.2–9.7)	9.7 (9.1–10.3)	10	0.14
Mon 3	1.7 (0.9–2.4)	2.4 (1.5–3.2)	1.7	0.28	2.6 (2.1–3.0)	3.3 (2.8–3.7)	4.0	0.06	9.5 (8.6–10.5)	10.2 (9.4–11.0)	10	0.29
Mon 4	2.4 (0.8–4.0)	3.5 (2.2–4.8)	4.3	0.32	2.6 (1.9–3.3)	3.7 (2.9–4.5)	4.0	0.06	10.3 (9.0–11.6)	11.1 (9.7–12.6)	13	0.44
Mon 5	3.3 (1.3–5.2)	3.7 (2.7–4.8)	4.3	0.63	3.2 (2.4–4.0)	3.6 (2.9–4.2)	4.0	0.51	11.4 (9.0–13.8)	11.6 (10.7–12.6)	13	0.83
Mon 6	3.5 (1.5–5.5)	4.7 (3.6–5.8)	4.3	0.22	3.2 (2.3–4.2)	4.0 (3.4–4.7)	4.0	0.14	11.2 (9.3–13.1)	11.9 (10.8–13.0)	13	0.44

	Mean Iron (mg)				Mean Zinc (mg)				Mean Selenium (ug)			
Mon 7	6.5 (3.5–9.5)	6.9 (5.1–8.8)	7.8	0.78	4.8 (3.7–5.9)	4.7 (3.9–5.6)	5.0	0.88	15.6 (11.1–20.0)	12.7 (10.0–15.4)	10	0.22
Mon 8	6.7 (1.4–12.0)	6.9 (3.7–10.2)	7.8	0.91	4.5 (3.6–5.4)	4.6 (3.6–5.7)	5.0	0.86	13.8 (6.7–20.9)	13.9 (11.6–16.2)	10	0.97
Mon 9	8.0 (1.1–17.1)	8.7 (5.8–11.7)	7.8	0.75	4.6 (3.7–5.5)	5.4 (3.9–7.0)	5.0	0.38	12.6 (3.4–21.9)	15.6 (10.7–20.4)	10	0.37
Mon 10	6.6 (–8.0–21.3)	9.4 (5.8–13.1)	7.8	0.34	5.1 (4.4–5.7)	5.8 (3.6–8.0)	5.0	0.67	14.6 (–6.0–35.1)	15.4 (11.0–19.7)	10	0.81
Mon 11	10.2 (5.3–15.1)	7.0 (3.9–10.1)	7.8	0.10	6.2 (4.5–7.8)	4.9 (2.4–7.4)	5.0	0.33	17.6 (2.5–32.7)	13.7 (8.9–18.4)	10	0.17
Mon 12	9.5 (–11.5–30.4)	8.2 (6.1–10.3)	7.8	0.46	5.5 (2.0–9.0)	5.7 (3.5–8.0)	5.0	0.85	18.7 (–41.9–79.2)	15.0 (10.0–20.0)	10	0.39
Mon 13	8.6 (–18.9–36.1)	6.8 (5.7–7.9)	7.8	0.27	5.9 (–1.2–12.9)	5.1 (3.9–6.3)	5.0	0.31	19.6 (12.9–26.3)	19.7 (16.3–23.0)	10	0.97

Values are mean monthly mineral intakes and 95% CI for symptomatic and control infants. RNI stated where one exists.

Table 5.12 Mean vitamin intake per day for dietary intake prior to diagnosis for symptomatic infants and their controls.

	Mean Vitamin A (ug)				Vitamin C				Mean Vitamin E (mg)		
Mon	**Symptomatic (95% CI)**	**Control (95% CI)**	**RNI (µg/day)**	**P**	**Symptomatic (95% CI)**	**Control (95% CI)**	**RNI (mg/day)**	**P**	**Symptomatic (95% CI)**	**Control (95% CI)**	**P**
Mon 1	476.4 (453.8–499.0)	483.8 (466.4–501.3)	350	0.61	38.3 (32.3–44.3)	37.8 (34.1–41.5)	25	0.88	3.7 (2.8–4.6)	3.6 (3.1–4.2)	0.85
Mon 2	521.7 (496.2–547.2)	544.3 (532.6–600.0)	350	0.06	38.9 (32.9–44.9)	45.4 (39.7–51.0)	25	0.15	3.8 (2.7–4.9)	4.5 (3.6–5.4)	0.34
Mon 3	536.7 (503.1–570.3)	568.1 (546.1–590.2)	350	0.11	43.7 (36.4–51.0)	48.9 (42.3–55.5)	25	0.33	4.4 (3.1–5.8)	4.8 (3.9–5.8)	0.63
Mon 4	554.9 (452.4–657.4)	654.0 (547.7–760.3)	**350**	0.23	49.2 (38.3–60.0)	61.5 (47.4–75.6)	25	0.24	5.1 (3.1–7.2)	5.9 (4.6–7.3)	0.49
Mon 5	666.4 (514.5–818.3)	662.4 (616.0–708.8)	350	0.95	54.0 (41.7–66.3)	61.8 (53.4–70.3)	25	0.28	5.2 (3.5–7.0)	6.3 (5.1–7.6)	0.31
Mon 6	807.4 (436.8–1178.0)	797.4 (696.0–898.8)	350	0.94	63.2 (49.0–77.5)	73.2 (62.3–84.0)	25	0.26	6.5 (4.2–8.7)	6.9 (5.6–8.2)	0.70
Mon 7	1210.8 (313.7–2107.8)	929.6 (751.6–1107.6)	350	0.34	71.5 (54.7–88.2)	85.5 (72.1–99.0)	25	0.18	11.6 (0.6–22.5)	6.9 (5.5–8.3)	0.18
Mon 8	723.5 (521.4–925.6)	1032.4 (594.9–1469.8)	350	0.28	68.3 (51.6–85.1)	71.5 (53.9–89.1)	25	0.79	5.9 (2.1–9.8)	5.5 (4.5–6.5)	0.68

	Mean Vitamin A (ug)				Vitamin C				Mean Vitamin E (mg)		
Mon	**Symptomatic (95% CI)**	**Control (95% CI)**	**RNI (µg/day)**	**P**	**Symptomatic (95% CI)**	**Control (95% CI)**	**RNI (mg/day)**	**P**	**Symptomatic (95% CI)**	**Control (95% CI)**	**P**
Mon 9	790.2 (120.4–1700.7)	881.3 (602.7–1159.9)	350	0.68	70.5 (2.9–138.1)	73.3 (48.2–98.4)	25	0.88	13.6 (6.7–33.8)	5.5 (4.2–6.8)	0.04
Mon 10	731.0 (124.0–1338.1)	818.3 (570.5–1066.1)	350	0.69	81.8 (13.9–149.7)	77.1 (41.2–112.9)	25	0.88	16.2 (1.3–31.1)	5.9 (4.0–7.8)	0.12
Mon 11	717.9 (119.7–1316.1.5)	729.6 (446.5–1012.7)	350	0.94	84.0 (18.2–149.9.)	56.5 (28.6–84.3)	25	0.21	14.6 (1.8–27.4)	4.9 (1.9–7.9)	0.19
Mon 12	568.1 (56.8–1080.2)	747.3 (622.1–872.4)	350	0.09	108.9 (32.9–184.9)	61.1 (51.2–71.0)	25	0.09	28.2 (2.7–51.7)	5.5 (3.1–7.8)	0.20
Mon 13	642.8 (71.6–1214.0)	638.4 (350.7–926.0)	350	0.98	100.2 (27.6–172.8)	52.3 (34.5–70.1)	25	0.01	32.6 (3.3–61.9)	4.4 (2.4–6.3)	0.19

Values are mean monthly vitamin intakes and 95% CI for symptomatic and control infants. RNI stated where one exists.

5.6 The relationship between an infant's specific feeding pattern and food allergy development by the age of two years

5.6.1 Whole diet analysis by Principal Component Analysis (PCA)

The first step of carrying out the PCA on the infant diet was to select the variables within the data on which to carry out the analysis. Together these variables need to reflect an infant's complete diet as accurately as possible and so their selection needs to be appropriately considered. For this study the variables included in the model were selected by using i) data from previous infant feeding studies and ii) observations made during infant food diary coding.

Data from previous studies into infant nutrition (33;173;230), and studies looking at early nutrition and allergy development (12;14;231), provided information as to what foods are important markers of early dietary intake and what foods are thought to be important in early allergy development. Additionally, observations made when entering food into the excel database (section 4.2.1.2.1.1) were also used when deciding which variables to include in the analyses. These observations were mostly to do with markers of dietary patterns. For example, at the time of the study, broccoli was not included in any commercial baby foods so if it was in the recorded diet, then it was a marker of home prepared foods. Conversely, butternut squash was regarded as a marker of a commercially prepared food as it was seldom prepared and cooked by mothers. Hummus marked a diet that included food which the infant could feed themselves and is a common constituent of an infant who is being fed by the 'baby-led weaning' infant feeding practice. Potato products, cook-in-sauces and ready meals were markers of on-going diets that contained commercially produced, 'adult' convenience foods. By including these variables an idea of an infant's diet could be determined without including all the food eaten in a week in the analysis.

Since the infant diet can change greatly over a short period of time, two separate analyses were run, one looking at characteristics of the early diet such as duration and exclusivity of breastfeeding, timing and types of solid food introduced and the overlap between introduction of solids and breastfeeding. The second analysis looked at the on-going infant diet after solid introduction, incorporating such characteristics as type of foods eaten, diversity of the diet, commercial versus home prepared infant foods, and 'healthy' versus 'unhealthy' weaning foods. As this second pattern was looking at the on-going infant diet, it could not include foods that could be affected by an infant's food allergy (since this would could be a source of error) and so foods

containing moderate to large amounts of dairy, egg, wheat, fish, peanut and tree nuts were not included in the analysis. In addition, special dietary foods such as hydrolysed formulae and soya products were not included in the analysis.

5.6.1.1 Patterns of early infant diet.

In the PCA of variables related to the early infant diet, 8 principal components had an initial Eigenvalue above 1.00 and these components accounted for 73% of the variance (Table 5.13). Correlation matrices for this analysis are given in Appendix 10 (Table A10.1).

Looking at the scree plot (figure 5.6) and the data in table 5.13, the first 5 components which accounted for 62 % of the variance observed were identified for further analysis (Table 5.14). The first component, which accounted for 25% of the observed variance was characterised by infant nutrition, predominantly breast milk. The main characteristic for the second component was solid food introduction. Those foods associated with being introduced earlier in the process of solid introduction into the infant diet (e.g. baby rice) had a higher score than those foods introduced later in the process (e.g. eggs). The third component's main characteristics were related to the intake of egg, fish and wheat. The fourth component was characterised by commercial baby foods and the fifth by formula milk. After the analysis was run each infants resultant score for each component was saved as a variable and then a Mann-Whitney U test was carried out to determine if there was a difference in pattern scores between the symptomatic infants and their controls. There were no significant differences between the two experimental groups for any of the 5 early infant diet factors ($p=0.248$; $p=0.109$; $p=0.959$; $p=0.845$; $p=0.184$ respectively).

Table 5.13 Eigenvalues and percentage variance for first eight components for early infant diet identified by Principal Component Analysis on all infants (n=123).

Component	Initial Eigenvalues		
	Total	% of Variance	Cumulative % of variance
1	7.876	25.406	25.406
2	5.186	16.728	42.135
3	2.618	8.445	50.579
4	1.843	5.944	56.524
5	1.552	5.005	61.529
6	1.319	4.256	65.785
7	1.163	3.750	69.535
8	1.050	3.387	72.922

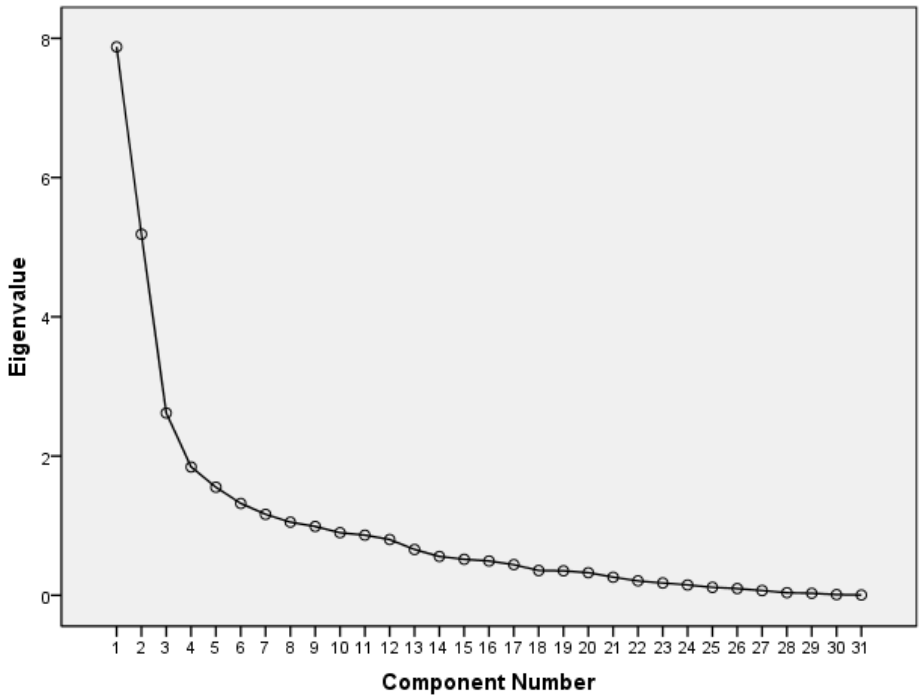

Figure 5.6 Scree Plot for early infant diet Principal Component Analysis on all infants (n=123).

Table 5.14 Five components for early infant diet identified by PCA on data from all infants (n=123).

Variable included in the analysis	Component				
	1	2	3	4	5
Duration of soya and breast milk overlap	.430	-.117	.115	.103	-.124
Duration of fish and breast milk overlap	.898	.108	-.316	.038	.140
Duration of wheat and breast milk overlap	.955	.062	-.057	-.039	.067
Duration of egg and breast milk overlap	.855	.144	-.120	-.010	.077
Duration of any milk and breast milk overlap	.667	.202	-.002	-.103	.626
Breastfeeding duration	.933	.172	.062	.032	.133
Duration of breast milk and any solid overlap	.884	.007	.095	.081	.105
Duration of breast milk and infant formula overlap	.490	.235	.094	.020	.699
Age infant first had any solids	.095	.887	.010	.076	.055
Age infant first had Hummus	-.002	-.011	-.019	-.046	.001
Age infant first had any fish	-.091	.155	.913	.058	.002
Age infant first had wheat	-.031	.298	.302	.207	.178
Age infant first had any Baby Cereal	.087	.891	.015	.034	.088
Age infant first had commercially prepared savoury baby food	.082	.390	-.064	.629	.022
Age infant first had commercially prepared	-.016	.205	.033	.786	-.015

sweet baby food					
Age infant first had yogurt/Fromage Frais	.130	.539	.282	.162	.146
Age infant first had avocado	.020	.323	.067	-.056	-.055
Age infant first had carrots	.068	.868	.160	.059	.051
Age infant first had lentils	.001	.333	.275	.196	.090
Age infant first had any apples	.177	.842	.130	.225	-.020
Age infant first had banana	-.028	.721	.100	.179	-.025
Age infant first had strawberry	.148	.476	.231	-.144	-.138
Age infant first had any cows' milk protein	.691	.038	.109	.228	-.492
Age infant first had egg	.166	.056	.510	-.049	-.102
Age infant first had cow's milk ingredient	.069	.454	.137	.354	.081
Age infant first had oily fish	-.028	.184	.711	.026	.068
Age infant first had white fish	-.098	.114	.881	.034	.007
Age infant first had bread	-.023	.194	.264	.055	-.019
Age infant first had tinned baked beans	.036	-.198	.075	.445	.458
Age infant first had infant formula	.727	.018	.082	.097	-.473
Age infant first had blueberry	.097	.171	.086	.653	-.136

Rotation Method: Varimax with Kaiser Normalization. Rotation converged in 8 iterations.

5.6.1.2 On-going infant diet patterns

In the PCA of variables related to the on-going infant diet, 6 principal components had an initial Eigenvalue above 1.00 and these components accounted for 72% of the variance (Table 5.15). Correlation matrices for this analysis are given in Appendix 10 (Table A10.2).

Figure 5.7 shows the scree plot for this analysis and details of the first six components are given in Table 5.16. Two of the components were significantly different between the two experimental groups (Mann-Whitney U: Component 1 p=0.002; Component 3 p=0.017). These two components both indicated dietary patterns associated with the intake of fruit and vegetables, poultry, oily fish, ready meals, potato products and cook-in sauces. In the first component, positive scores were associated with the intake of fruit, vegetables, poultry and fish and low/negative scores were associated with potato products and ready meals. For component 3, positive scores were associated with potato products, ready meals and cook-in sauces with low/negative values being assigned to fruits and vegetables. In both cases the mean score for the control infants was associated with the 'healthier' pattern whereas the mean score for the symptomatic infants was associated with the less healthy dietary pattern (Table 5.16).

Table 5.15 Eigenvalues and percentage variance for first six components for on-going infant diet identified by Principal Component Analysis on all infants (n=123).

Component	Initial Eigenvalues		
	Total	% of Variance	Cumulative % of variance
1	12.129	40.431	40.431
2	2.261	7.538	47.969
3	2.055	6.851	54.921
4	1.530	5.101	64.787
5	1.460	4.866	69.215
6	1.328	4.428	72.472

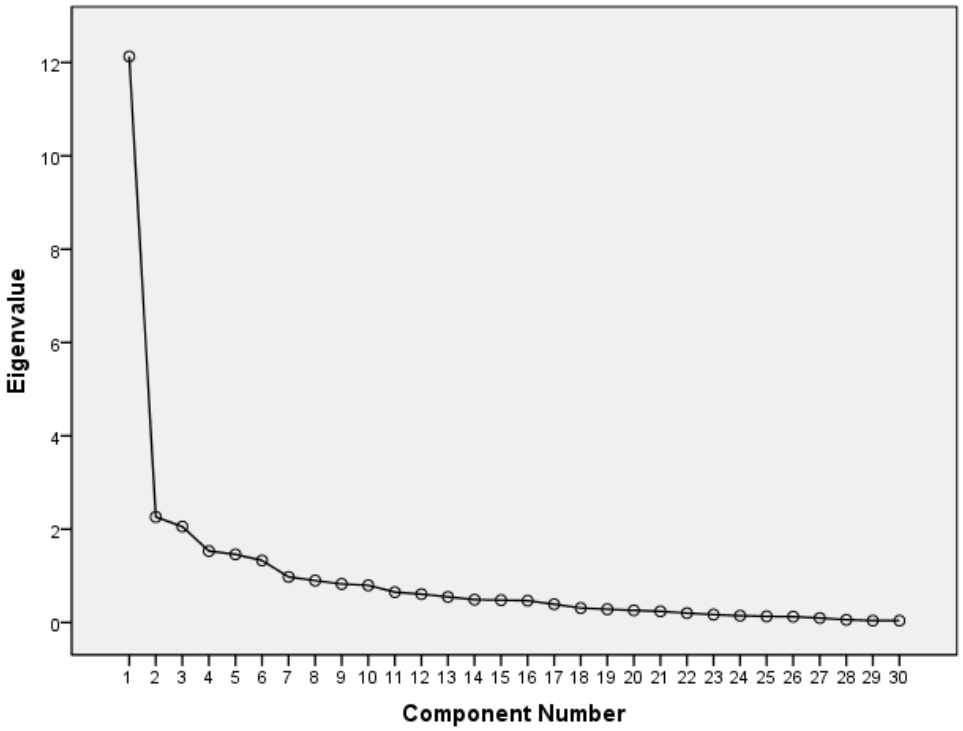

Figure 5.7 Scree Plot for on-going infant diet Principal Component Analysis on all infants (n=123).

Table 5.16 Six components for on-going infant diet identified by PCA on data from all infants (n=123).

Variable included in the analysis	Component					
	1	2	3	4	5	6
Breastfeeding duration	.106	.142	.061	.000	.702	-.021
Age of infant at introduction of solids	-.102	-.106	-.012	-.023	.799	-.089
Number of weeks apples were included in the infants diet	.904	.225	-.024	.062	-.084	.053
Number of weeks infant had commercially prepared savoury baby food	.705	-.108	.158	.460	-.094	-.069
Number of weeks infant had commercially prepared savoury baby food more than once a day	.280	-.142	-.015	.791	-.030	-.137

Number of weeks infant had commercially prepared sweet baby food	.673	.038	.020	.429	-.137	-.049
Number of weeks infant had commercially prepared sweet baby food more than once a day	.022	.131	.131	.860	.029	.111
Number of weeks pizza was included in the infants diet	.130	.802	.082	.018	-.108	-.230
Number of weeks potato products were included in the infants diet	.112	-.184	.759	-.074	-.026	-.098
Number of weeks ready meals were included in the infants diet	.114	.220	.631	.282	.027	.209
Number of weeks cook-in-sauces were included in the infants diet	.084	.177	.767	.069	.068	.029
Number of weeks carrots were included in the infants diet	.907	.183	.089	.109	-.042	.127
Number of weeks onions were included in the infants diet	.861	-.016	.164	-.009	.054	.055
Number of weeks potatoes were included in the infants diet	.864	-.016	.143	.007	.003	.032
Number of weeks peas were included in the infants diet	.688	.212	.160	.107	.081	-.150
Number of weeks lentils were included in the infants diet	.506	.307	-.087	-.095	.256	.162
Number of weeks banana was included in the infants diet	.878	.226	.074	.121	-.058	.115
Number of weeks peaches were included in the infants diet	.620	.208	.135	.067	.104	.112
Number of weeks raspberries were included in the infants diet	.711	.275	-.122	.148	.119	.164
Number of weeks strawberries were included in the infants diet	.788	.150	.026	.119	-.063	.233
Number of weeks oily fish was included in the infants diet	.637	.230	.358	-.165	.078	.123

Number of weeks broccoli was included in the infants diet	.737	.230	-.077	.102	.045	-.037
Number of weeks beef was included in the infants diet	.878	-.018	.243	-.019	-.044	.015
Number of weeks poultry was included in the infants diet	.920	.120	.130	.091	-.017	-.018
Number of weeks Marmite was included in the infants diet	.267	.528	.302	.147	-.212	.427
Number of weeks jam was included in the infants diet	.277	.050	.133	-.042	-.187	.746
Number of weeks chocolate was included in the infants diet	.328	.344	.379	.043	-.256	-.565
Number of weeks dried fruit was included in the infants diet	.301	.561	-.056	-.262	.267	.227
Number of weeks toddler packet snacks were included in the infants diet	.582	.481	.042	-.023	.112	-.055
Number of weeks fruit (not pureed) was included in the infants diet	.399	.553	.192	.162	.277	.236

Rotation Method: Varimax with Kaiser Normalization. Rotation converged in 13 iterations

Further PCAs related to the on-going infant diet were run with the final analysis having 7 principal components with an initial Eigenvalue above 1.00, these components accounting for 69% of the variance (Table 5.17). Correlation matrices for this analysis are given in Appendix 10 (Table A10.3).

Table 5.17 Eigenvalues and percentage variance for first seven components for final Principal Component Analysis on the on-going infant diet for all infants (n=123).

Component	Initial Eigenvalues		
	Total	% of Variance	Cumulative % of variance
1	9.639	32.131	32.131
2	2.830	9.434	41.565
3	2.417	8.058	49.622
4	1.815	6.052	55.674
5	1.445	4.817	60.492
6	.1.317	4.391	64.883
7	1.156	3.853	68.736

Looking at the scree plot (figure 5.8) and the data in table 5.17, the first 3 components which accounted for 50 % of the variance observed were identified for further analysis (Table 5.18). Component 1, which accounted for 32% of the variance, depicted a dietary pattern with positive values associated with a healthy infant diet that was predominantly home cooked and low/negative values associated with highly processed adult foods (such as ready meals, cook in sauces, pizza and bacon) and the use of commercial baby foods more than once a day. This could be described as a diet that follows infant feeding guidelines (33). Component 2 is a dietary pattern defined by finger foods, with the highest positive values allocated to healthy finger foods and the low/negative values allocated to pureed baby foods and unhealthy finger foods. The third component is defined by high values being allocated to highly processed adult foods (Table 5.18).

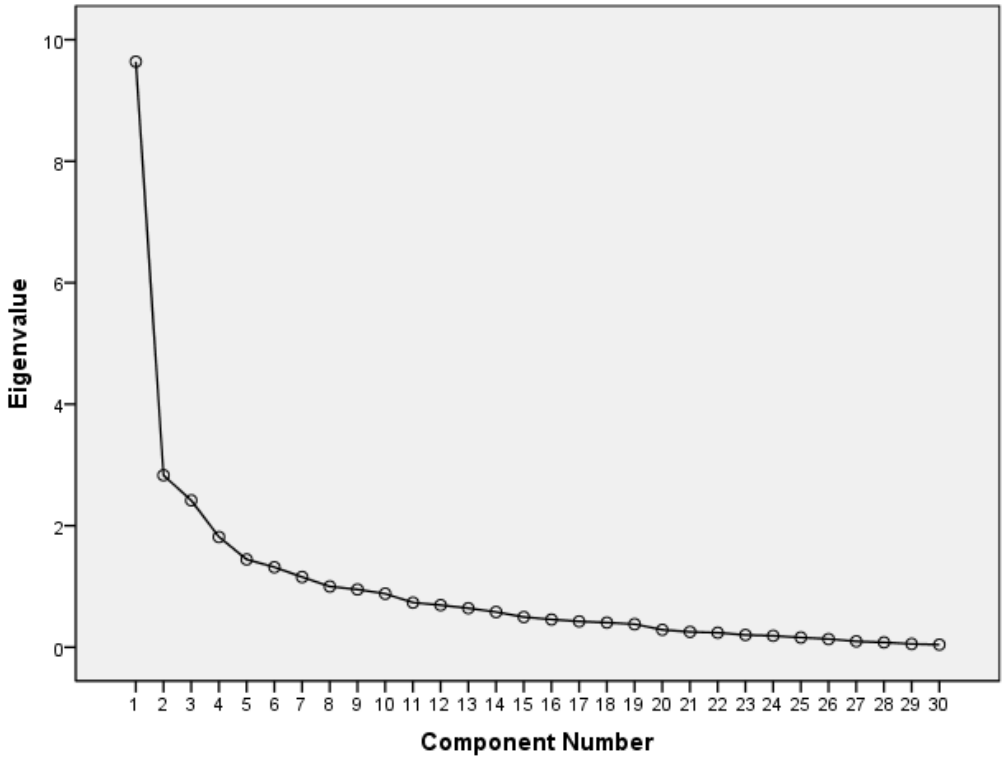

Figure 5.8 Scree Plot for final Principal Component Analysis on all infants (n=123) for on-going infant diet

Table 5.18 Three components for on-going infant diet identified by final Principal Component Analysis on data from all infants (n=123).

Variable included in the analysis	Component		
	1	2	3
Number of weeks infant had commercially prepared savoury baby food	.716	.174	-.082
Number of weeks infant had commercially prepared savoury baby food more than once a day	.253	.016	.01
Number of weeks infant had commercially prepared sweet baby food	.730	.039	.063
Number of weeks infant had commercially prepared sweet baby food more than once a day	.074	.089	.106
Number of weeks 'fast food' was included in the infants diet	.364	.487	.124

Number of weeks potato products were included in the infants diet	.048	.661	-.047
Number of weeks ready meals were included in the infants diet	.021	.551	-.055
Number of weeks cook-in-sauces were included in the infants diet	-.067	.755	.191
Number of weeks avocado was included in the infants diet	.289	-.092	.344
Number of weeks broccoli was included in the infants diet	.724	.066	.218
Number of weeks carrots were included in the infants diet	.883	.167	.193
Number of weeks apples were included in the infants diet	.892	.102	.264
Number of weeks grapes were included in the infants diet	.183	-.030	.824
Number of weeks bacon was included in the infants diet	.053	.083	-.103
Number of weeks poultry was included in the infants diet	.853	.194	.169
Number of weeks sausages were included in the infants diet	.267	.775	.137
Number of weeks Marmite was included in the infants diet	.298	.270	.190
Number of weeks jam was included in the infants diet	.220	-.052	.175
Number of weeks dried fruit was included in the infants diet	.173	.012	.749
Number of weeks toddler packet snacks were included in the infants diet	.539	.165	.377
Number of weeks raw fruit was included in the infants diet	.303	.162	.696
Number of weeks oily fish was included in the infants diet	.548	.367	.273
Number of weeks ‘sweeties’ were included in the infants diet	.167	.046	-.004
Number of weeks crisps were included in the infants diet	.228	.597	-.163
Number of weeks bread was included in the infants diet	.637	.349	.396
Number of weeks butternut squash was included in the infants diet	.760	-.096	.064
Number of weeks parsnips were included in the infants diet	.749	.039	-.002
Number of weeks sweetcorn was included in the infants diet	.516	.038	.258
Number of weeks kiwi was included in the infants diet	.308	.002	.094
Number of weeks oranges/citrus were included in the infants diet	.331	.169	.399

Rotation Method: Varimax with Kaiser Normalization. Rotation converged in 8 iterations.

The mean scores for component 1, 2 and 3 were .000. The mean score for component 1 was significantly different between the symptomatic and control infants (p=0.002)) (Table 5.19).

Table 5.19 Mean scores for three components identified from final PCA analysis on all infants (n=123).

	Symptomatic (n=41)	Control (n=82)	P value §
Mean score for component 1	-.390 (Range -1.467 to 1.918)	.185 (Range -1.467 to 2.202)	0.002
Mean score for component 2	-.064 (Range -.828 to 2.872)	.030 (Range -1.136 to 5.406)	0.589
Mean score for component 3	-.143 (Range -1.711 to 2.882)	0.068 (Range -1.504 to 4.003)	0.758

§ Mann-Whitney U test

5.7 Combined analysis of variables linked to development of allergy

The aim of this analysis was to further investigate the variables which were identified in previous sections as being significantly different between the symptomatic and control infants. Such analyses can establish whether any of these variables are still associated with a particular outcome when the effects of other variables are controlled for. Also the relative risk of each variable on allergy outcome can be calculated from which the effect of any change in behaviour can be established.

For this analysis the variables were included in an overall binary logistic regression analysis. The variables included in the first analysis were 'full' maternal diet during pregnancy, age at introduction of any solid food, age of introduction of cows' milk ingredient, duration of overlap between any milk containing foods and breast milk, duration of overlap between cows' milk infant formula and breast milk, and a 'infant guideline' diet score (as determined by PCA). However assessing for high correlations between these variables indicated low tolerance scores for age solids were introduced, age at introduction of cows' milk ingredient, duration of overlap between any milk containing foods and breast milk, and duration of overlap between cows' milk infant formula and breast milk indicating these variables are correlated with other

variables in the model. The age of introduction of cows' milk ingredient and duration of overlap between cows' milk infant formula and breast milk were removed from the model which resulted in acceptable tolerance scores for the remaining variables in the model (Table 5.20)

Table 5.20 Collinearity statistics for variables included in binary logistic analysis.

Model	Collinearity Statistics	
	Tolerance	VIF
Full maternal diet	.968	1.033
'Infant guidance' score from PCA analysis	.946	1.057
Duration of overlap between any milk and breast milk	.893	1.120
Age infant first received solids	.857	1.167

Dependent Variable: Allergy outcome

All remaining variables were entered into the model in the same block and at the same time by using 'forced entry' methodology, with the 'full' maternal diet variable and 'maternal education' variables being identified as categorical and the remaining variables as continuous. Table 5.21 presents the results for the binary logistic analysis for the comparison between cases and controls. Statistical significance was based on whether the 95% CI included unity or not. Based on this analysis it can be seen that the age at solid introduction and 'infant guideline' weaning diet had a statistically significant effect on allergy outcome with both variables having a protective effect (Table 5.21). The 'healthfulness' of the weaning diet had the greatest protective effect with a relative risk of 0.577 with the variable describing the increasing age of solid introduction into the infant's diet having a small protective effect with a relative risk of 0.847. The χ^2 for the model was 12.9 with a significance level of p=0.024 with the model correctly classifying 73% of infants.

Table 5.21 Results of logistic analysis for assessment of variables associated with food allergy development.

Variable Name	Variable type and coding	B (SE)	Relative risk	95% CI for Relative Risk
'Full' maternal diet	Categorical. Full maternal diet =0; Avoiding any food =1	-1.361 (0.725)	0.256	0.062-1.062
Age at solid introduction	Continuous. Unit change =week	-0.166 (0.070)*	0.847	0.738-0.972
Duration of any milk and breast milk overlap	Continuous. Unit change =week	0.010 (0.023)	1.002	0.892-1.124
'Infant guidelines' diet score	Continuous. Arbitrary units	-0.550 (0.268)*	0.577	0.341-0.976
Maternal age	Continuous. Unit change = 1 year	0.002 (0.059)	1.002	0.892-1.124
Maternal Education	Categorical. Education after 18 years of age =1 Education up to and including 18 years =0	-0.331 (0.904)	0.719	0.122-4.229

*Model χ^2 = 12.9, p=0.024; *p<0.05*

6. Discussion

This study set out to determine whether specific aspects of infant feeding in the first year of life were associated with the development of food allergy by two years of age. Infants who developed food allergy, defined using food challenges, were compared with age matched controls. All infants were part of a larger cohort study.

There were four study hypotheses focussing on different aspects of infant feeding in the first year of life. These were as follows:

1a. Children who develop food allergy by two years of age will have a 5% lower rate for the initiation of breast feeding than children who do not develop a food allergy

1b. Mean breastfeeding duration for children who develop food allergy by two years of age will be 4 weeks less than for children who do not develop a food allergy

1c. Mean exclusive breastfeeding duration for children who develop food allergy by two years will be 4 weeks less than for children who do not develop a food allergy

1d. Mean duration of concurrent breastfeeding with any cows' milk protein for children who develop food allergy by two years will be 4 weeks less than for children who do not develop a food allergy

2. Children who develop food allergy by two years of age will have solid foods introduced into their diet a mean of 4 weeks earlier than children who do not develop a food allergy

3. Children who develop food allergy by two years of age will have a 0.5 standard deviation difference between the mean intake of energy, protein, fat, iron, zinc, selenium, vitamin A, vitamin C and vitamin E than children who do not develop a food allergy

4. Children who develop food allergy by two years of age will have a 0.5 standard deviation difference in their PCA pattern score than children who do not develop a food allergy.

The mothers of the children kept weekly diaries of all foods consumed and breast milk and infant formula consumption.

For hypothesis 1, none of the predetermined differences were met; the difference in breastfeeding initiation was only 3.6%; the difference for mean duration of

breastfeeding and exclusive breastfeeding was 2.2 and 0.04 weeks respectively; and the mean duration of concurrent breastfeeding was 0.96 weeks. For hypothesis 2, the difference in mean age at solid introduction was 1.36 weeks. For hypothesis 3, for a number of months for a number of nutrients there was a difference between the groups of 0.5standard deviation or more but it was only different for 6 months or more for Zinc and Vitamin E (see Appendix 9). For hypothesis 4 there was a difference of 0.6 standard deviations for the pattern score identified as being significantly different between the groups.

Due to the lack of differences between the groups, and therefore sufficient power in the analysis, the null hypothesis for hypothesis 1, 2 and 3 (except for zinc and vitamin E) cannot be rejected. For hypothesis 3, since there was no difference between the groups for all months, the null hypothesis cannot be accepted or rejected. For hypothesis 4 the null hypothesis can be rejected.

Despite the lack of powering in the analyses to reject the null hypothesis for study hypotheses 1, 2 and 3, the study did demonstrate some specific aspects of infant feeding were associated with the development of food allergy. Introduction of any type of solid food before 17 weeks of age was associated with food allergy development with neither the type nor the nature of the food having any additional affect. Breastfeeding was not associated with food allergy development *per se*, but if the infant received breast milk at the same time as any cows' milk it appeared to protect against the development of allergy to any type of food. An infant diet that was age appropriate, predominantly home-made, contained large amounts of fruit and vegetables and low levels of adult, highly processed foods was found to be protective against food allergy. Additionally, a maternal diet that did not exclude any food appeared to be protective against the development of food allergy. However, these variables may not be acting independently of each other as previous research has shown the quality of the maternal diet influences the quality of the infant diet (33) and also that complementary feeding influences breastfeeding duration (173). Additionally, all these variables have been shown to be associated with external factors such as maternal age and education (33;173). Because of the possibility of some of these variables not acting independently of each other or of external confounders, a logistic analysis containing all these variables was carried out and will be considered and discussed in section 6.6.

The macro or micronutrient of the infant diet was not found to be associated with the development of food allergy but the small numbers in these analyses, the multiple tests carried out means these findings cannot be considered scientifically robust.

Finally, non-food intake found to be associated with the development of food allergy were anti-reflux medication during pregnancy and anti-inflammatory medication during lactation.

This chapter discusses these main findings in detail and will be followed by a critical consideration of the strengths and limitations of the whole study.

6.1 The role of complementary feeding in food allergy development

6.1.1 Infant formula introduction

The use of infant formula has long been associated with the development of food allergy (145) but in this study there was no association demonstrated between the age formula was first introduced and allergy development (p=0.478) although this analysis only had a power of 8% (see Appendix 8). There was a significant difference (p=0.000) between the groups for the duration that infant formula was given, with control infants being given formula for longer (mean 21 weeks) than the symptomatic infants (mean 12 weeks). However, this difference is due to 'reverse causality' (where an observed effect is due to the outcome measure, not the cause of it) since cow's milk allergic infants have cows' milk, and therefore standard infant formula, removed from their diet. If these infants are removed from the analysis then the difference between the groups is no longer significant.

Looking at the use of infant formula containing prebiotic which may help prevent food allergy development due to altered infant gut flora (162;232), 39% of infants who went on to develop a food allergy used infant formula containing prebiotic compared to 61% of infants who did not develop a food allergy. This difference was not statistically significant (p=0.094) but the power of this analysis was only 68% (see Appendix 8). There did appear to be a significant difference between the groups when looking at duration of prebiotic consumption; but again this result is due to 'reverse causality' in that specialist formulae taken by infants diagnosed with a food allergy do not contain prebiotics. As before, when the cows' milk allergic infants are removed from the analysis, the difference is no longer statistically significant.

6.1.2 Solid food introduction

The relationship between the introduction of solids into an infant's diet and the later development of allergy is of major interest to any researcher looking at risk factors of food allergy development (19;65;189). Consequently, the prospective food

diary data collected for the infants involved in this study provides a unique opportunity to investigate the relationship between solid introduction and allergy development.

Even for this cohort of 123 well-educated older mothers, the mean age of solid introduction was 20.3 weeks, with 114 (93%) of mothers not meeting the recommendation to introduce solids at 26 weeks or later (172;206). Further analysis of the timing of any solid introduction demonstrates that in addition to not meeting the recommendation for the timing of solid introduction, mothers did not adhere to the advice to introduce food according to developmental readiness, not according to age (201). This is demonstrated in Figure 5.2 by the peaks in the number of infants who first had solids at ages relating to monthly milestones (12, 17 and 21 weeks). Additionally, there was a peak at 25 weeks suggesting some mothers were aiming for the recommended age of 26 weeks to introduce solids but they did not quite manage to delay the introduction of solids to this age.

Analysis of the diary data shows that infants who did not go on to develop a food allergy were introduced to solids later than infants who did develop a food allergy. Despite the difference being statistically different (p=0.044), the difference in median age at solid introduction at one week was small. However, by looking at the range of ages when solids were introduced into the infant diet, it could be seen that the range was wider for control infants compared to symptomatic infants (Table 5.4). Consequently, it may be an oversimplification to state that the later the timing of solid introduction the better. The prospective nature of this study's infant food diaries enabled the percentage of symptomatic infants introduced to solids at each week compared to control infants to be examined (Figure 5.4). From this it can be seen that the time period where there is a big difference between the groups is between 12 and 16 weeks. During this period 15% of symptomatic infants were introduced to solids but only 9% of control infants were introduced to solids during this time. *Post hoc* analysis demonstrated that when the data was categorised to introduction of solids at 16 weeks of age or earlier compared to 17 weeks and older, the difference between the groups was significant (Chi square p=0.011). If the analysis is carried out with the age categorised into age at solid introduction 20 weeks or less compared to age at solid introduction 21 weeks or more, there was no significant difference between the groups. This implies the period of interest where the introduction of solids increases the risk of allergy is up to 17 weeks of age (4 months).

It is of particular interest to be able to identify the time period where solid introduction may heighten the likelihood of a food allergy developing. This has often been spoken of theoretically but has not yet been identified. From analysis of findings of different studies into solid introduction and allergy development this crucial time period could be after 4 months of age (80;177), between 4-6 months of age (182;192)

and before 9 months (193). It would be interesting to be able to establish if there is an age range where solids need to be introduced to reduce allergy risk. To date there has been no recommendation of when solids should be introduced by to reduce allergy but recent literature suggests that delaying the introduction of solids into the infant's diet increases allergy risk (181-183;185;186;192;194). Unfortunately, there were insufficient numbers introducing solids beyond 26 weeks in this study cohort to establish if there was an upper age limit beyond which solid introduction may heighten allergy risk. However, it is possible to look at the data for the introduction of cows' milk as an ingredient and this could act as a surrogate measure for solids *per se*. Figure 5.5 shows the difference in the introduction of cows' milk as an ingredient between the two study groups occurs at two timeframes: before 20 weeks and from 28 weeks onwards. A stratified analysis of the timing of cows' milk as an ingredient shows the time span of 29 weeks and above isn't associated with either a lower or higher allergy risk (p=0.166). However, giving cows' milk as an ingredient before 20 weeks was associated with the increased risk of allergy development (p=0.008) implying it is the earlier time period where the effect is made.

An additional analysis that could be carried out to see if there is an upper age limit before which solids should be introduced to reduce allergy risk would be to look at whether delayed introduction of a food heightens allergy risk. This can be done with this data for the introduction of egg as an ingredient into the diet. The data from this study shows median age of introduction of egg as an ingredient is higher in symptomatic infants than control infants, but that this difference is not statistically significant (p=0.067). However, as has been explained above, the power of this study is inadequate so this association may reflect a mechanistically important relationship. *Post hoc* power analyses show the effect size of this association to be 0.46 of a standard deviation with a 75% powering at an α level of 5%. Whilst this effect may be due to reverse causation, the effect size suggests this finding may be of potential interest for allergy prevention programmes and so represents an area for further research.

Research into the introduction of solids and allergy development has suggested the mode of delivery of the allergen in food may be an important factor in food allergy development (192;233). This is due to the change in allergenicity of proteins according to how they are cooked/processed (234;235). Data from this study did demonstrate allergy to be associated with introduction of cow's milk as an ingredient but not in all forms. However, a food processing/cooking effect was not demonstrated for egg or for any other food, although this may be due to a lack of power in the analysis since the relationship was approaching statistical significance for egg as an ingredient (p=0.067) but not as 'egg' (p=0.930), with a power calculation showing that 174 infants would be

needed for this analysis to be adequately powered (Appendix 8). In this study, cows' milk as an ingredient represented foods such as savoury cheese dishes and sweet desserts such as rice pudding and yogurt. The 'any cow's milk' criteria included whole cow's milk either as a drink or when used on breakfast cereal. Since whole cows' milk was introduced later to symptomatic infants (due to reverse causality) this is likely to compensate for the effect seen for cows' milk ingredient. Consequently, the difference in the form of cows' milk taken and allergy development demonstrated by this data cannot be said to be causal.

Until recently it was advised that for allergy prevention, the introduction of solid food be delayed until six months and that allergenic foods should be introduced into the infant diet even later (236). This implied that allergenic foods had a more potent allergenic effect than non-allergenic foods. Consequently, the timing of the introduction of only allergenic foods to the whole cohort of 123 infants was analysed to see if there was a difference between the groups; there was no difference ($p=0.665$). Since food allergy is an allergen specific condition, it is possible that the timing of specific allergenic foods may have an effect on the development of an allergy to that particular food. To address this question, analyses were stratified for the timing of the food to which each infant became allergic to. No significant difference was seen between the groups except the association previously seen for cows' milk as an ingredient, but this lack of association may be due to the very small numbers in these sub-analyses leading to a situation where the power was insufficient to obtain statistically significant results.

Overall, these findings suggest it is the timing of solid introduction that is important with power analyses showing the effect size of the observed feeding practice difference of 2 weeks to be 0.3 of a standard deviation which is considered to be a 'moderate' effect (228). Solid introduction before 17 weeks appears to promote sensitisation whereas solid introduction after 17 weeks seems to promote tolerance. This implies that the mechanism which is acting post 17 weeks of age is acting upon non-allergen specific tolerogenic mechanisms of the infant immune system since the foods introduced at this time are not considered allergenic (apart from cows' milk). The mechanisms acting at this time may be via a variety of epigenetic (237;238) and immunological (65;76) mechanisms and the foods that are introduced during this time may be delivering the substrates required for the induction of such processes. These foods are commonly fruit and vegetables. These foods have been shown to modify immune responses in older children (10;190) with the proposed mechanism being due to their antioxidant action on inflammatory processes (239) and Treg cells (187). It is not a quantum leap to hypothesise these foods have a similar effect in infants.

The implication of this study's findings on the timing of solid introduction and the development of allergy adds to the on-going discussion regarding adherence to the WHO and DoH feeding recommendations to exclusively breastfeed for 26 weeks (11;240). The WHO advice is designed to reduce infant mortality and morbidity rates (23), particularly for infants born in developing countries, but the relevance of this advice for infants born and brought up in the UK has already been brought into question (11). These study findings do not question the recommendation to continue to breastfeed, ideally for at least the first year (172) or two years (206) of life, but it does add some additional data to the argument which questions the necessity for breastfeeding to be exclusive beyond four months of age, since analysis of this study data did not show an additional protective effect of delayed solid introduction beyond 17 weeks of age.

Whilst it is important that developed countries support infant feeding recommendations directed at low and middle income countries, a one size fits all recommendation may not deliver reduced disease risk to all populations. The public health question to ask in each context is does the benefit of exclusive breastfeeding to 26 weeks outweigh any potential increased risk of developing food allergy? To establish the big picture effect of changing the recommendation for solids to be introduced before 26 weeks of age, risk calculations need to be made to establish how many infants would have an increased risk of disease (particularly gastro-intestinal disease) compared to the reduced numbers of infants developing food allergy. In the meantime, since only 7% of women manage to exclusively breastfeed to 17 weeks of age (173), there needs to be a continued effort to encourage mothers to exclusively breastfeed for as long as they can manage, and ideally for 26 weeks as per the current recommendation (172).

6.2 The role of breast milk in food allergy development

For this study, the breastfeeding initiation rate was 95% compared to a national initiation rate of 78% reported in the 2005 Infant feeding Study (IFS) (173). As demonstrated in Figure 6.1, the age demographic of the mothers involved in the parent PIFA study differed from that of the IFS study. As maternal age has been shown to affect breastfeeding initiation (241-243), this difference in breastfeeding initiation rates may be due to the different age demographic or may be a reflection of previously observed increasing breastfeeding rates (173). Consequently, the data were adjusted for maternal age and the breastfeeding initiation rate for the adjusted sample was 86%. This suggests the difference is not purely due to the different maternal age profile between the two study samples. However, it is not appropriate to extrapolate data

from a sample of n= 123 to enable comparison with a sample of n= 8210, particularly as there was only one mother in the lowest age range of <20 years in this study sample. Therefore for this dataset, it is only possible to report breastfeeding initiation, not to draw conclusions as to what this data may mean as regards allergy outcome on a population level.

However, breastfeeding initiation rates were not significantly different between the two experimental groups, neither was duration of breastfeeding or duration of exclusive breastfeeding (Table 5.3). However, these analyses are inadequately powered with a powering of11%, 7% and 15% respectively (see Appendix 8). A Kaplan Meier survival analysis also did not show a statistical difference in breastfeeding survival patterns between the groups (Figure 5.1) but this analysis suggests that mothers may change how they feed their infants with the diagnosis of allergy. Consequently, consideration of the diet in relation to the timing of food allergy diagnosis needs to be made in the subsequent interpretation of any analyses on the differences in feeding behaviour between the groups to ensure an observed difference in diet happened before symptoms appeared. This will ensure causality is not wrongly applied by establishing that the behaviour of interest occurred before the outcome (212).

As is the case with other studies looking at breastfeeding practises and allergy outcome in unselected cohorts (130-134;136;244), this study showed no protective effect of breastfeeding on allergy outcome. In addition to the under powering for this analysis, critics may also argue this finding is due to poor exclusive breastfeeding duration (median duration of exclusive breastfeeding was 8 weeks for the study cohort of 123) which is considerably different to the WHO and DoH recommendation of 26 weeks (172;206). However, there is debate over the necessity for breastfeeding to be exclusive for it to convey its health benefits (11), at least in developed countries where the risks of infections from unclean water and poor sanitation may be considerably lower. The number of study infants still receiving breast milk at 26 weeks (32%) was higher than for the IFS (26%) but is still only a third of all study infants. Consequently, the lack of an association may be due to insufficient study infants receiving breast milk for a long enough period of time for a protective effect to be seen. This lack of association may also be due to differences in breast milk composition, particularly of factors involved in the development of the infant's adaptive immune system namely maternal immunoglobulins, oligosaccharides, cytokines, glycoproteins, LCPufas, lysozyme, and nucleotides the level of which were not measured in this study.

Further analysis was carried out on the relationship between breast milk and the development of allergy by stratifying the analysis for the food the symptomatic infants became allergic but there was no difference between the experimental groups

although for peanut allergy the difference (mean of 9 weeks for peanut allergic infants compared to mean of 20 weeks for control infants) did approach statistical significance (0.05). This observation could not be anything to do with an overlap effect between the infant receiving breast milk and the allergen (as presented in section 5.6) since no peanut allergic infant or control infant received peanut whilst still being breastfed. It may be due to a general protective role of breast milk as proposed by Hoppu et al (41) but if that is the case, why wasn't the effect seen for all foods, which would be expected due to increased numbers in the analysis?

6.2.1 Maternal medicinal intake during lactation

In the observed difference for breastfeeding duration and peanut allergy development, it is possible that prolonged breastfeeding was a marker for something else which may have an effect on allergy outcome such as the general health of the mother. Conversely, short breastfeeding duration could be an indication of maternal disease such as infection with the intake of medication acting as a marker. Table 5.1 shows that although percentage antibiotic intake whilst breastfeeding was higher amongst mothers whose infants subsequently developed food allergies (39%) compared to mothers of infants who did not (24.4%), this difference was not statistically significant (p=0.349) although the power of the analysis was only 52%. A *post hoc* analysis of medicine consumption (paracetomol and non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroid, diabetes and asthma medication and medication for reflux) during breastfeeding showed mothers of infants who subsequently developed a food allergy took more anti-inflammatory medication than mothers of infants who did not develop a food allergy (44.4% compared to 24.2% p=0.017). Intake of all other medication was not significantly different between the groups although as is the case for the other baseline analyses, these tests were underpowered (see Appendix 8). Further stratification of this analysis investigating NSAID use and food allergy development showed that mothers of infants who developed milk, egg, and peanut allergy all took more anti-inflammatory medication whilst breastfeeding than those mothers of infants who breastfed but whose infants did not develop a food allergy (p=0.018, p=0.040 and p=0.040 respectively).

NSAIDs work by inhibiting the action of the enzyme cyclo-oxygenase (COX) (245). Inhibition of COX results in heightened allergic inflammation with increased production of cytokines which promote the allergic response (246;247). NSAID's are minimally transported into breast milk (248) so their mode of action on allergy outcome is likely to be due to an altered cytokine profile of the mother's milk (caused by the action of COX) as it has been previously shown that cytokines found in breast milk can affect allergic outcome in the infant (42;43;45). Breast milk samples were not collected from study mothers so comparison of the cytokine profile of breast milk from the two study groups to confirm this hypothesis.

6.2.2 Breastfeeding and concurrent complementary feeding

Due to the presence of a number of immunomodulatory factors in breast milk, it is thought to play an important role in the development of oral tolerance. However there is no overwhelming evidence of the protective effect of breast milk on allergy development. Some reviews reporting beneficial effects (9;126;127) and others report no such effect (5;128). It has been proposed that this may be due to the difference in composition of breast milk between mothers (85;144). However, another explanation for the apparent conflicting findings of the role of breast milk on allergy development is that the immunomodulatory role of breast milk is only apparent when the immune system is exposed to antigen at the same time as breast milk (185;249).

Data from this study shows a protective effect on allergy development when cows' milk protein from any source was present in the infants diet at the same time as breast milk (p=0.047) and this relationship was also seen for infant formula and breast milk (p=0.047) with an effect size of 0.22 standard deviation. The relationship was still present even if the continuous overlap variable was recoded into a categorical variable of overlap or no overlap of any cows' milk and breast milk (p=0.015) and overlap of infant formula and breast milk (p=0.022). Additionally, *post hoc* analysis showed no effect for when the overlap took place (p=0.300).

It was not possible to see if this association could be seen between the groups for any other allergenic food since there were insufficient numbers in the two groups to run the analyses. For example, only 11% of the 123 infants in the thesis study had egg introduced into their diet whilst they were still receiving breast milk. This figure was higher for fish (22%) and wheat (33%) but it still represents low numbers overall. This is because wheat, egg, fish, and the other allergenic foods are generally introduced after 26 weeks of age, as per DoH recommendation (201), by which time 2/3rds of mothers had stopped breastfeeding.

Breastfeeding initiation and duration have increased over the last decades, as has the mean age at which solids, particularly allergenic ones, are introduced into the infant diet (173). Both these changes are due to amended infant feeding recommendations (24;172). The net result of these changes is that the length of time infants receive solids whilst still being breastfed has reduced. This has happened over the last few decades when the incidence of food allergy is reported to have increased. Consequently, population-wide and allergy specific infant feeding recommendations may have had a role to play in the increase in food allergy rates. Indeed, a number of countries and specialist bodies have recognised this possibility and amended their infant feeding recommendations to incorporate the potential role of concurrent breastfeeding on food allergy development (250;251) whilst other professional bodies have called for further research into the topic (118;119). The findings from this study

add support to the possible protective effect of concurrent breastfeeding on allergy development.

In summary, this study has not been able to demonstrate a protective effect of breast milk *per se* but it has added to the argument that concurrent breastfeeding with the introduction of allergenic proteins into may be protective against the development of food allergy.

6.3 The role of the whole infant diet and food allergy development

A number of nutritional/dietary variables may be acting on the development of food allergy in infants so focussing on one or two dietary characteristics may be an over-simplification of the complex interactions taking place. It is for such situations that principal component analysis has been used in the past to describe dietary patterns that may be affecting disease outcome (197).

From the findings of this study, it seemed that there were two distinct types of dietary factors that may be affecting allergy outcome. One was to do with early infant feeding incorporating such factors as breastfeeding, duration of breastfeeding, duration of breastfeeding overlap with allergenic foods and age at introduction of different foods whilst the other was to do with the nature of the on-going infant diet. Consequently, the analysis to look at the whole infant diet was split into two aspects: the early infant diet and then the on-going infant diet. The variables in the early infant diet pattern included those that described breastfeeding, age and nature of solid introduction, duration of concurrent feeding of allergenic foods and breast milk, and the age at which solids which described the nature of the diet (e.g. foods used in home cooking, commercially produced baby foods) were first given. PCA analysis on these variables identified the first 5 components which made up 62% of the variance observed. The first component was characterised by infant nutrition, predominantly breast milk. The main characteristic for the second component was solid introduction. The third component's main characteristics were related to the intake of egg, fish and wheat. The fourth component was characterised by commercial baby foods and the fifth by formula milk. There was no difference between symptomatic and control infants in how they 'scored' for these five patterns demonstrating that these identified feeding patterns did not have an association with the later development of food allergy.

However, the findings for the pattern analysis for the on-going infant diet did show a difference between the two study groups. This analysis looked at on-going diet to try to determine whether there was any evidence of a continuing immunomodulatory effect of the diet on allergy development. The variables incorporated into this PCA analysis gave a picture of the type of diet the infant was eating. Was it predominantly home cooked or did it include commercial baby foods and if so were they eaten on a regular basis? How diverse was the diet? Did the diet include 'adult' commercially prepared foods? Where fruit and vegetables eaten often and when they were eaten were they in a processed or unprocessed form? However, the potential for incorrectly identifying an observed difference in dietary intake between the groups as causal is heightened once the on-going diet was looked at. Therefore, no diet characteristic that could be an effect of a dietary change due to a food allergy diagnosis was incorporated into the pattern. As a consequence, no detail of dairy, egg, wheat, fish, seed or nut containing foods were included in the analysis.

The first PCA on the on-going infant diet identified two patterns for which symptomatic and control infants had significantly different scores ($p=0.015$ and $p=0.030$ respectively). The first pattern had high scores for foods that were age appropriate (commercial baby foods, toddler snacks, carrots, potatoes and bananas) and lower scores for foods which could be considered to be an 'adult' food (potato products, ready meals and cook-in-sauces). In the third pattern, high scores were given to 'adult' foods and low scores to foods which could be considered to be 'healthy' (such as dried fruit, lentils and broccoli). Since there were some similar elements between these two patterns, further analyses were run to incorporate the two patterns into one. This was done and the mean scores for the symptomatic infants were significantly different from those for the control infants for the first component which accounted for 32% of the variance ($p=0.002$). This analysis had 92% power (Appendix 8). This component depicted a dietary pattern with positive values associated with an infant diet that was predominantly home cooked with high intake of fruit and vegetables and low/negative values associated with highly processed adult foods (such as ready meals, cook in sauces, pizza and bacon) and the use of commercial baby foods more than once a day. A pattern similar to this has been identified in previous research into infant dietary intake and was described as the 'infant guidelines' pattern (33).

Despite not including variables which may lead to 'reverse causality' in the analysis, it is still necessary to consider the results in light of the fact that some of the observed differences in mean scores may be due to food allergy rather than a cause of it. However, food allergy sufferers are more likely to consume home-prepared foods of low allergenicity as part of their allergy management plan (252). Therefore, these findings that food allergic infants had a diet which contained more processed adult

foods and less fruit and vegetables are unlikely to be a result of food allergy, and may actually be a cause of them.

Although there has been much research into infant diet and allergy outcome, to date, no study has looked at the whole infant diet. Some studies have looked at the timing of important feeding events during infancy (157;183;231;253), and others have looked at the link between diet and existing allergic disease in older children and adults (190;254;255) but not how the whole infant diet may be affecting the development of allergic disease. Consequently, the findings from the PCA analysis of the infants included in this study have provided a new insight into how the infant diet may modify allergy development.

6.4 The role of maternal diet in food allergy development

6.4.1 Nutrient supplement use

No differences were found between the two experimental groups for the intake of any dietary supplement during either pregnancy or lactation (Table 5.1). The fact there was no difference in maternal multi-vitamin, folic acid, LCPufa's and vitamin D supplement use during pregnancy or lactation would seem to imply these nutrients have no role to play in the development of an infant food allergy. However, this would almost certainly be an over-simplification and go against the emerging data (10;76;80;83;98). As has been mentioned throughout this discussion, this thesis study was underpowered to detect small but potentially important associations in the development of food allergy. Appendix 8 shows the powering of the analyses into maternal nutrient supplement use and allergy outcome was very low, ranging from 5% to 21% so a potentially significant association could have been found had the study numbers been higher.

In this study cohort of 123 mothers, less than 15 % took vitamin D or LCPufa's supplements and although notably more mothers of control infants took LCPufa's (17% compared to 10%), this difference was not statistically significant ($p = 0.591$). Very few study mothers took vitamin D supplements during pregnancy (2.4%) or lactation (3.3%) despite supplementation being a DoH recommendation (256). Consequently, the lack of a statistical difference between the groups may be a consequence of a lack of power in the analysis as much as a lack of a specific nutrient effect on allergy outcome.

Multi-vitamin use was more common with 23.5% of mothers taking them during lactation and 57.7% during pregnancy but this still equates to less than 2/3rds of mothers taking multivitamins during pregnancy. As stated earlier, there was no

difference between the groups for multi-vitamin consumption but once again this finding may be due to insufficient numbers taking multi-vitamins during pregnancy and to a lack of statistical power in the analysis due to the relatively small study sample size to be able to detect anything but a strong experimental effect. Additionally, when the fact that multivitamins taken by mothers consist of a variety of different 'over-the-counter' products whose composition vary considerably is also taken into account, then it can be seen that actual intake for each micro-nutrient could differ greatly between mothers who took a multi-vitamin. This could lead to a multi-vitamin consuming group which is too heterogeneous to analyse together without diluting the evidence of an effect of any particular nutrient.

Folic acid intake during pregnancy approached 90% of all 123 mothers. Although the percentage of mothers of infants who did not develop a food allergy who took folic acid supplements was lower than for mothers of infants who did go on to develop a food allergy (84.1% compared to 87.8%), this difference was not statistically significant ($p=0.144$). Again the data may not allow for adequate sensitivity to determine any biological effect occurring as questionnaire responses were only categorised as to whether the mother took folic acid or not. However, some mothers took folic acid just for the first months of pregnancy whereas others took it throughout pregnancy. The timing of folate intake may be crucial in terms of gene expression which is the proposed mechanism for its effect on allergy outcome (108); the method of categorising the data may have resulted in the evidence for this effect being lost. Analysing the data again but this time taking into account timing of folic intake showed 43% of mothers whose infants later developed a food allergy took folic acid throughout pregnancy but only 26% of mothers whose infants did not develop a food allergy took folic acid throughout pregnancy. However, this difference was not statistically significant ($p=0.144$) but as stated previously, this may be due to a lack of statistical power in the analysis to detect anything but large differences between the two study groups so a possible association should not be ruled out.

As a whole, looking at maternal supplement use in this cohort of 123 mothers has provided some interesting discussion points but no significant differences were found between the two experimental groups. However, this may be due to insufficient statistical power in the analysis due to small study numbers. Using the data available from all the EuroPrevall birth cohorts, it would be possible to look at maternal supplement use for all nine trans-European cohorts which would give enough power to determine even small differences in : i) supplement use, ii) heterogeneity of multi-vitamins used, and iii) timing differences in supplement use.

6.4.2 Maternal food avoidance

There was a difference between the experimental groups as to whether the mother avoided any foods during pregnancy (Table 5.1). Data looking at maternal food avoidance during pregnancy and lactation and the development of food allergy in the infant was collected by way of the PIFA questionnaires. From the responses, a categorical variable of whether the mother avoided any food was calculated. Only 9.8% of mothers of symptomatic infants ate a full diet during pregnancy compared to 26.8% of mothers of control infants and this difference was significant ($p=0.032$) with an odds ratio of 3. The foods most commonly avoided for both groups were peanuts, tree nuts and shellfish. Other frequently avoided foods were eggs, seeds and legumes.

Prior to 2009, high numbers of mothers avoided allergenic foods, particularly peanuts, during pregnancy and to a lesser extent during lactation (257) in accordance with DoH advice of 1998 (169). This advice was directed at reducing the risk of an infant developing a food allergy although adherence to it has been questioned (257;258). The advice itself was amended in 2009 after the evidence base was revisited and evidence supporting a beneficial effect of mothers avoiding allergenic foods during pregnancy and lactation was not considered strong enough to continue to recommend avoidance (120). It appears the data from this study supports the thinking behind the amended advice that avoidance for allergy prevention during pregnancy should no longer be recommended. However, the mechanisms which may be associated with these findings are not clear. The observed associations may be due to an *allergenic* effect of dietary avoidance or a *nutritional* effect of dietary avoidance. Avoiding allergenic foods during pregnancy has in the past been thought to reduce the risk of food allergy developing in the infant by preventing the immune system coming into contact with allergenic foods 'too early' (12). However, due to the lack of evidence for a protective effect of such measures, avoidance during pregnancy is no longer recommended (118;119). Indeed, it is possible that avoidance may actually promote allergy development, as implied by this study's findings, due to depriving the immune system of necessary antigenic triggers which promote the development of tolerance.

Alternatively, the mode of action to explain these findings may be due to dietary avoidance leading to a maternal diet which no longer provides the nutrients required for the normal development of the immune response in the infant. The foods routinely avoided by mothers in this study are a good source of at least one nutrient implicated to have a role in the development of food allergy (10;70), namely LCPufa's, zinc, vitamins A, D and E.

Nevertheless, just because a mother's diet lacks a food which is a good source of a nutrient, it is inappropriate to assume her particular diet contains lower levels of the

nutrient in comparison to the diet of a mother who is eating the food in question. An analysis of a detailed food diary would be required to do that, but since the data on maternal diet for this study was obtained from a questionnaire that only asked about the intake and avoidance of certain foods and food groups, such an analysis is not possible as part of this study. It would be possible to do sub-analyses of the data from the whole EuroPrevall study to see if identification of a culprit food group could be made, but this would not be able to shed light on the mechanisms involved; for this, food diary data is required.

6.4.3 Maternal medicinal intake during pregnancy

Analysis of medicine consumption (paracetomol, NSAIDs, corticosteroid, diabetes and asthma medication and medication for reflux) during pregnancy showed mothers of infants who later developed a food allergy took anti-reflux (antacid) medication more frequently than mothers of control infants (28.6% compared to 12.3% p=0.01). Intake of other medication did not differ significantly between the groups but since the powering of these analyses were weak (Appendix 8), the possibility of a significant association cannot be ruled out.

The use of antacids has been linked with the development of food allergy due to findings from studies using mouse models and using epidemiological data (259;260) although an observational study looking at antacid use and the development of allergy in the offspring found no association (but an association was found between antacid use and later development of asthma) (261). The data from this thesis study adds more weight to the suggestion that antacid use in pregnancy may increase the likelihood of food allergy development in the offspring. With the large numbers who suffer from reflux during pregnancy (thought to be between 30-50% of pregnant women (262)), the potential role of antacid use and allergy development needs to be further investigated. The current proposed mechanism is related to antacid consumption leading to less efficient digestion of foods, in particular proteins, resulting in larger peptide molecules being taken up into the bloodstream. These larger peptides are more capable of sensitizing the immune system (259).

6.5 The role of macro and micro nutrients in allergy development

Despite the link between nutrient intake and immunological function being well established (76), nutrient intake in the infant as determined by prospective food diary

has not been studied to date. Using diary data collected as part of this thesis study, the relationship between infant nutrient intake and subsequent allergy development was assessed but no difference in nutrient intake was found for any macro-or micro-nutrient. However, infants that went onto develop an allergy had a lower intake of zinc than control infants for weeks 8, 12 and 16 but the difference was not statistically significant (p=0.09, p=0.06 and p=0.06 respectively). However, the power of these analyses was only 51%, 66% and 54% (see Appendix 9) suggesting that with larger numbers a significant association may be seen. Zinc deficiency is thought to effect immune function by leading to decreased cytokine production and skewing the immune system to produce an allergy promoting environment (263). However, despite it being demonstrated that zinc deficiency supresses the induction of oral tolerance in rats, only a weak positive association has been demonstrated between zinc intake and allergy outcome in children (10).

For the time-points where these differences were seen, the infants were still predominantly receiving either breast milk or infant formula so the observed difference could be due to the different composition data for breast milk and infant formula in the database (0.3 mg/100ml for breast milk, 0.4 mg/100ml for formula). This difference does not seem large but over the course of one day it could lead to a difference as great as 1.0 mg. There was no statistically significant difference in breastfeeding rates between the experimental groups but that does not rule out a difference in zinc intake. Nevertheless, without knowing the actual composition of mothers' milk we cannot assess actual intake accurately. These findings point towards an interesting association between infant zinc intake and allergy outcome. This could be further investigated with studies which are adequately powered to detect differences in nutrient intake and also collect breast milk samples which can then be analysed to determine actual micronutrient composition.

Despite the results of this study showing no statistically significant association between an infant's nutrient intake and the later development of allergy, the possibility of there being an association cannot be ruled out for a number of reasons. Firstly, the numbers involved in this analysis were relatively small and also decreased with the infants' age, leading to a lack of statistical power in the analysis. Secondly, since the actual nutrient profile of each mothers' breast milk was not known, standard values from the data base were used therefore any difference in the nutritional content of individual mothers' milk was not taken into account. Finally, the data available for breast milk, infant formula and solids eaten did not differentiate between n-3 and n-6 PUFA content of food so it could not be established whether there was a difference in the intake of these particular fats between the groups. Consequently, from this data it cannot be stated that infant nutrient intake is not related to allergy outcome.

6.6 Combined analysis of variables linked to development of allergy

Since infant dietary intake is effected by many factors, including maternal diet, maternal age, and maternal education level (33), it is possible that the variables shown in the previous sections to be associated with the development of infant food allergy are themselves interrelated with each other or another factor such as maternal age or education. A logistic regression analysis would be able to identify which of these variables was having an effect on their own by removing any potential confounding effect of the others. Six variables were included in the logistic analysis (age at solid introduction, full maternal diet, breast milk and cows' milk from any source overlap and healthy on-going infant diet, maternal age and maternal education).

The analysis showed that the age at solid introduction and the 'healthfulness' of the diet (as determined by the 'infant guideline' dietary pattern) have an independent association with the development of food allergy. The analysis showed healthfulness of the infant diet has the most effect on allergy outcome with an increase in 'infant guideline' score reducing the risk of developing a food allergy by 46%. However, it is hard to describe specifically what an infant guideline score of 1 equates to. Instead a description of the dietary features positively associated with the dietary pattern needs to be given. These features are a diet that is age specific, that regularly incorporates fruit and vegetables either from homemade or commercially made baby foods (but with commercially prepared baby foods being only given occasionally), and with predominantly lean and not processed protein sources.

The logistic analysis also showed age at solid introduction had a significant effect on allergy outcome with each week's delay in solid introduction reducing the risk of the infant developing a food allergy by 15.5%. However, this may be an oversimplification of the situation because when age at solid introduction is looked at independently, there was an upper limit for this effect of 17 weeks.

According to the logistic analysis, concurrent breastfeeding and whether mothers ate a full diet during pregnancy no longer had a significant effect on allergy development in the infant.

6.7 Strengths and Limitations of the study

6.7.1 Strengths

The main strength of this study is that it used prospectively collected food diaries to record the diets of the infants included in the study. This was the first study to capture such prospective data and because diet was recorded in the present it was more likely to reflect true intake. However, perhaps more importantly, the diet data was collected before the person recording the diet knew the allergy status of the child. The diet was thus not likely to be biased, although it was subject to random error, and reporter burden may have led to some foods being missed, or there may be the possibility that foods the reporter 'knew' the child should not eat were avoided. The key point here though is that these factors are not likely to be different between cases and controls as these data were collected (for the most part) prior to diagnosis. These factors could lead to random error but not bias which would affect the estimates of the effect of diet on the development of food allergy.

Previous studies into the relationship of the infant diet and food allergy development have assessed the infant diet by using a recall questionnaire (182;192;264). Data collected in this way may be subject to recall bias, particularly if the person recording the diet knows the allergy status of the infant, because when dietary intake is recorded after a child has been defined as having food allergy it is very likely that the person recording the child's past diet will recall it differently from a person whose child has not been defined as food allergic. This recall bias may lead to a false over estimate of the effect of foods thought by the reporter to cause the allergy. (This behaviour has been recorded for other conditions, for example women with breast cancer over recall their fat intake compared with known past consumption and in comparison with non-breast cancer controls (265). In other circumstances it could be that a person underestimates consumption if they feel guilty about what they fed their child and which they thought might have caused the symptoms.

In addition to collecting prospective food diaries, prospective symptom diaries were also collected to ensure the timing of first symptoms of food allergy could be accurate, to reduce the risk of reverse causality.

Another strength of this study is the clear, unambiguous diagnosis of food allergy by what is considered to be the gold standard diagnostic technique (DBPCFC) (198). In the field of food allergy, diagnostic criteria can be diverse and lead to a heterogeneous population, so a clear diagnosis is a key starting point when researching any aspect especially prevalence and risk analysis.

Finally, the unique use of the food diary data collected in this study has allowed for a diverse range of analyses to be used. Amongst these is PCA which has not previously been used to investigate the relationship between infant feeding and allergy development,

6.7.2 Limitations

It was not possible to assess the validity of the dietary method used in this study, and it is difficult to assess how this might have been done in free living infants. The only way to accurately assess the true intake was to have some method of blind observing behaviour (using hidden cameras, for example) or to ask the subject to live in a laboratory where there food was measured as it was prepared. Neither of these methods are practical. As stated above, as long as there was no bias in the assessment method (i.e that the method was not used differently in cases and controls) it is unlikely that differences reported here could be due to bias, although small differences which were not statistically significant may have been missed because of measurement error and small sample size for some exposures in some sub-sets of subjects.

If the difference between the groups for the variable of interest was small it may not be detected if not enough subjects have been studied. When this happens a null hypothesis is accepted incorrectly (a type 2 error). To ensure this doesn't occur, best practice is to design a study that will detect a difference between the groups. However, since this was a case control within a cohort study, subjects were not recruited directly into this study and since the parent study was a prevalence study, we did not know how many cases there would be. *Post hoc* analyses have shown the study was underpowered for all hypotheses except hypothesis 2 (if the difference between the groups was 4 weeks. This under powering needed to be considered in the interpretation of the statistical tests (see section 6.7.2.1.3).

6.7.2.1 Minimising Error

The dietary assessment methodology selected for this study was the prospective food diary as it would ensure, if completed correctly, that all the data required was available. The sources of potential errors in these records were reduced as much as possible by training all mothers how to complete the diet diary. This included advice on how to use household measures to record intake and, as quantitative data was only collected one week out of four and only used for the nutrient analysis, the error resulting from portion size estimation has been kept to a minimum. Mothers were also encouraged to write down what the infant ate at the time in order to minimise recall error and they were encouraged to keep the food diary close at hand to facilitate this.

Response error was reduced by explaining the importance of recording what was actually eaten rather than what the mothers thought the infant should be eating. To reduce coding errors and errors associated with the use of food composition tables, the same protocol was used for all study participants so any error would be the same for both groups. Furthermore, the same researcher carried out all the data processing, coding and analysis as per the protocol thereby reducing the likelihood of there being a difference between the two study groups. However, for the analysis of the quantitative data, three researchers coded the diet data and this was a potential source of error. To minimise this risk, a strict protocol for coding the dietary intake data and for using the food composition tables was used. To further minimise this source of error, the same database (which included data added in for this project) was used by all researchers.

In this study, controls could not be selected by their demographic background to minimise differences with the symptomatic infants, but background differences could be investigated to determine if there was any demographic differences between the groups which may be a source of error. There were no statistically significant differences for demographic background in this study (Table 5.1). However, due to the small study size, small significant differences in background demographics may not have been detected due to insufficient power in the analysis (see Appendix 8)

Despite efforts to reduce error, there were sources of error that could not be eliminated. For the nutrient analysis of the infants' diets, nutrients provided by breast feeding could only really be thought of as an estimate since we did not have composition data for each mothers' milk nor did we have feeding duration from all diaries. However, a standardised procedure using data from previously published studies (224) was used for both study groups, so only if breastfeeding rates between the groups were different would this lack of detail lead to an error. In this study, breastfeeding rates were not significantly different ($p=0.295$).

Another potential source of error concerned the maternal intake data. Unlike the infant intake data, this was not collected by either food diary or by validated FFQ but by standardised questionnaire. This asked if the mother ate a certain food group and if they did eat it, had they changed their intake during the period of interest. These questionnaires also asked about nutritional and medicinal supplement intake, asking what was taken and how regularly. The pregnancy data was either collected at the late stages of pregnancy or in the first few months after the birth. The data regarding intake during breastfeeding was also collected retrospectively at 12 months of age. Consequently this data should not be considered to be the same quality as that

available for infant dietary intake as it was subject to maternal 'coding' and recall bias which the infant data was not.

6.7.2.1.1 Baseline characteristics of study population

It is a common phenomenon that research studies/surveys tend to have a proportion of older/well-educated mothers than those seen in the population from which the study participants were drawn (173), so it is not a surprise that the demographics of the PIFA study population (n=1170) differed from the demographics of the community from which it was recruited (Figure 6.1). However, the infants in the two experimental groups of the thesis study (n=123) did not appear to differ significantly from each other for any demographic or environmental measurement (Table 5.1). Nevertheless, due to a small study size the significance of small differences may not have been identified. However, as the thesis study was a 'nested within a cohort, case control' study using these mother/infant pairs, the skewing of the PIFA cohort demographics will not affect analyses looking at differences between symptomatic infants and their controls which make up the thesis study population. It does, however, mean that any study findings need to be extrapolated to population levels with care (212).

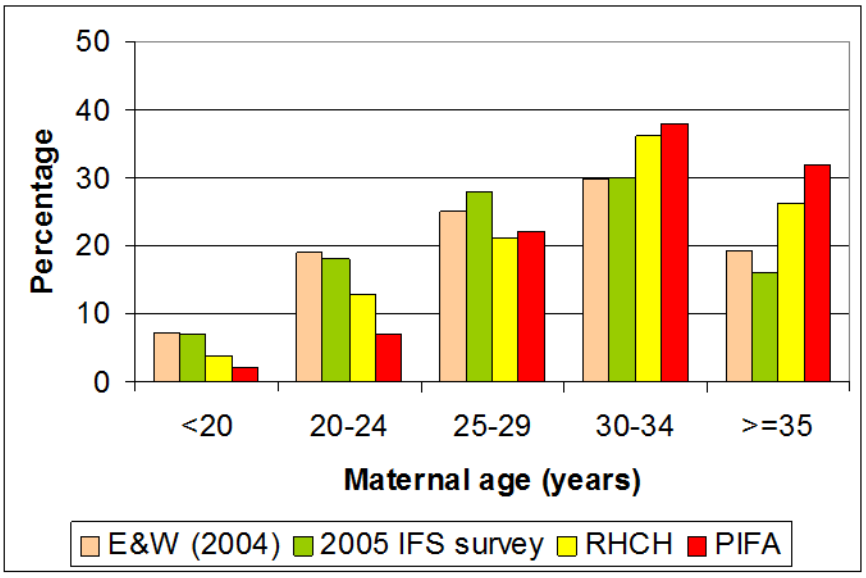

Figure 6.1 Comparison between maternal age in UK birth cohort and similar populations. *Percentage of mothers in each age range at enrolment into the UK birth cohort (PIFA) in comparison with England and Wales population; 2004 (E&W), 2005 infant feeding survey (IFS) ((173) and women delivering at Royal Hampshire County Hospital, Winchester (data from routine hospital data, 2007) (RHCH).*

6.7.2.1.2 Appropriate methodology

The food diary was considered to be the best method of dietary assessment for this study but if the diaries were not kept accurately they would not reflect actual intake. As was explained in section 3.3.3, determining the accuracy of the food record is difficult since any additional assessment on which to validate the diary data is impractical. Since the data was analysed in two different ways, the data may be considered accurate for one methodology but not the other.

Since the majority of the analyses for this study were concerned with the timing and frequency of feeding events, comparison against similar values from the infant feeding study can give an indication of whether the diet diaries gave valid data regarding the timing of major feeding events. However, for a correct comparison to be made the data from this study needs to be adjusted due to the different demographic profiles of the two study populations. The actual and adjusted values are shown in Table 6.2 along with the values from the 2005 IFS. The data shows that for breastfeeding at 6 months, and solid introduction at 4 months and 6 months the values are essentially the same. For breastfeeding initiation and duration, the rates are higher in the study cohort (n=123) but this is likely to be due to a continuation of already demonstrated improving breastfeeding rates (173) since this data was

collected between 2006 to 2008. Overall this comparison demonstrates that the diary data detailing dietary events delivered results comparable to those previously recorded.

Table 6.1 Actual and adjusted feeding characteristics and IFS data.

	Actual values for all infants (n=123)	Adjusted values for all infants (n=123)	2005 IFS values
Mean breastfeeding initiation rate	97%	86%	78%
Percentage infants receiving breast milk at 4 months	55%	47%	35%
Percentage infants receiving breast milk at 6 months	32%	27%	26%
Percentage infants receiving solids by 4 months	33%	50%	50%
Percentage infants receiving solids at 6 months	97%	99%	98%

The data was adjusted by altering the percentage of each of the age groups within the whole dataset to match that seen in the IFS and then used the age specific data to adjust each of the values.

To assess the relative validity of the dietary intake data for the infants nutrient intake, the mean energy intakes for the study population were compared to available standardised data (221) to see if they were significantly different from the published values. The results of the one way t-tests are detailed in Table 6.1 and show that for males, mean intake was significantly different from the recommended values for 7 out of 12 months and for females they were significantly different for 6 out of 10 months. In all these cases the mean energy intake was higher than that recommended.

Table 6.2 Table comparing recorded energy intake with published data of energy requirements for infants from 0-12 months of age.

Age in months (n)	Gender	Mean Energy Intake kcal/day (SD)	Energy Requirement (from Butte 2005 (221))	T test
1 (44)	Male	525 (51)	518	0.362
1 (32)	Female	500 (53)	509	0.367
2 (27)	Male	597 (43)	570	0.004
2 (23)	Female	566 (52)	556	0.367
3 (27)	Male	608 (74)	596	0.416
3 (23)	Female	593 (39)	585	0.317
4 (19)	Male	652 (87)	569	0.001
4 (19)	Female	650 (132)	537	0.015
5 (19)	Male	687 (126)	608	0.014
5 (19)	Female	643 (78)	600	0.028
6 (16)	Male	741 (127)	639	0.006
6 (15)	Female	684 (104)	626	0.049
7 (14)	Male	765 (97)	653	0.001
7 (8)	Female	769 (143)	628	0.027
8 (6)	Male	799 (131)	680	0.077
8 (4)	Female	763 (44)	651	0.014
9 (5)	Male	888 (91)	702	0.010
9 (2)	Female	874 (71)	673	0.156
10 (4)	Male	950 (171)	731	0.083
10 (2)	Female	996 (24)	695	0.037
11 (3)	Male	952 (137)	752	0.127
11 (1)	Female	1134	694	utc
12 (3)	Male	1280 (169)	775	0.035
12 (1)	Female	999	712	utc

This analysis can only give a very generalised indication of the accuracy of the food diary data since there are numerous variances intrinsic within the data such as unknown weight gain from month to month, the changing proportion of infants being breastfed as opposed to formula fed (since breastfed infants gain weight at a slower rate than formula fed infants) and the decreasing numbers across time.

In all but one instance, the reported energy intake was higher than the standardised value. However, the recommended values used (which were determined by new calculations), had shown that previous recommendations (which were set according to actual intake) were set too high, implying that actual intake is higher than the requirements (221). Since the values are higher than recommended, it could be suggested that these diaries were unlikely to be subject to under-reporting but no firm conclusion as to their accuracy for the calculation of nutrient intake can be made from these analyses.

6.7.2.1.3 Interpretation of statistical tests

This study was a case control within a cohort study where the infants were recruited to the parent cohort study investigating the prevalence of food allergy across Europe. As a result, an *a priori* sample size for this particular thesis study was not calculated before recruitment started. Additionally, when the study started, there was no pre-existing data available in the literature which could be used to carry out such calculations. At that time, studies looking at the relationship between the timing of solid introduction did not have DBPCFC diagnosed allergy as their outcome measure (177;181) and even now most do not (182;184;266). Also, there was no available data regarding the duration of concurrent breastfeeding and solid introduction, nor any research regarding dietary intake patterns in infants and allergy outcome. This is still the case. After completion of the study, *Post hoc* calculations were carried out using data from the study to determine what study numbers were required to have a power level of 90% at 5% significance and these demonstrated that for most of the hypotheses the study was underpowered (Table 4.1).

However, where the analyses had a statistically significant result, the sample size was adequate to establish that the observed difference is unlikely to have occurred by chance. However, where a difference was not significant, it may have been be due to a small sample size hence a power calculation was needed to be carried out to ensure accurate analysis of the statistical test. Power calculations on the maternal supplement use during pregnancy showed the power to detect an α level of 5% was only 5% for multivitamin use, 9% for folic acid intake, 21% for vitamin D intake and 9% for fish oil supplement use. For each of these variables, therefore, it cannot be stated

categorically that they do not have an effect on allergy outcome. Further research with larger numbers is required before this can be done.

For the timing of introduction of egg as an ingredient, the p value approached significance (p=0.067) and if the powering of the study was adequate the association may have been found to be statistically significant. A *post hoc* power calculation showed that in this analysis the power to detect significance at the 5% level was 65%. To have found a significant difference at the same effect level of 0.46 standard deviations would have required 174 infants (calculation using G Power statistical analysis package (267)).

However, for accurate interpretation of the statistical significance test, it is not just required that the analysis be adequately powered to ensure a type 2 error is not committed (ie a null hypothesis is accepted incorrectly) but also that a type 1 error is not made. Type 1 errors are when a null hypothesis is rejected incorrectly and occurs when multiple testing of the data occurs. In this study multiple testing of this nature occurred in the analysis of the baseline characteristics and the analysis into the relationship between the infant's nutrient intake and allergy outcome.

In summary, where the analyses found a statistically significant difference, these findings can be considered to be internally valid, since the two experimental groups were demographically similar, study methodology did not lead to bias, and there were enough subjects for the analysis to have sufficient powering for these variables despite the powering of the whole study being inadequate. However, since the whole study population is quite narrow in its demographic background, the study may not be externally valid and its findings need to be applied to broader populations with care. For example, total smoking rate in this population was low (6%) so it is not known whether these study findings are applicable to mothers' who smoke. This is particularly relevant given that maternal smoking is a known risk factor for infant allergy development (268). Again, only 24% of infants lived in an urban area and this may also have an effect on allergy outcome (269); the study findings may therefore not be applicable to infants who live in an urban area.

Nevertheless, due to the methodological strength of the data on which the analyses were collected, the study results can be reported with confidence but with the proviso that the work should be repeated for different population groups not represented in this study. In addition, for this study, reaching a statistically significant result cannot be seen as the 'be all and end all' due to the potential of both type 1 and type 2 errors. Instead, the results need to be considered in terms of the observed differences between the groups for the variables of interest and the effect size on

allergy outcome of these differences. This will then highlight this research study's most promising areas and indicate possible areas for further research.

7 Conclusions, implications and recommendations for future work

Food allergy has significant individual, household and societal costs (270;271) with the increasing prevalence of allergic disease a public health concern, particularly in Westernised, urbanised countries (272). Consequently there is great interest in the aetiology of food allergy in general, and the primary prevention of food allergy in particular.

This thesis study investigated maternal nutrition, breastfeeding practices, infant formula use and the nature of the infant diet in order to assess their relationship with food allergy development.

The main findings of this work were:

- A diet in the first year of life which follows current infant feeding recommendations (ie is predominantly home-made and well balanced, delivering healthy foods from the main food groups daily) may be protective against allergy development up to the age of two years.;

- Not introducing solids into the infant diet before 17 weeks of age may be protective against allergy development up to the age of two years;

- Breastfeeding whilst the infant is first introduced to allergenic may be protective against allergy development up to the age of two years (at least for cows' milk).

The protective nature of a healthy diet in the first year of life is a unique finding. To date there have been observations that healthy dietary patterns can modify allergy symptoms and even sensitisation rates in older children and adults (190;254;255) but this has not been demonstrated for the infant diet. The importance of introducing infants to a wide variety of home cooked foods containing plenty of fruit and vegetables has been part of infant feeding recommendations for many years but with the advent of more convenience foods in the homes, infants and young children are now consuming larger amounts of highly processed foods than ever before (273). These findings suggest that this change in infant and early childhood diets may be contributing to the increasing allergy rates. It may also be the reason for observed differences in infant allergy prevalence rates between European countries as demonstrated by the EuroPrevall study (unpublished data). For example allergy

incidence rates are higher in the UK, Holland and Germany where infants are fed a more processed diet, compared to rates in Italy and Greece where infants are still predominantly fed a traditional, home cooked weaning diet. Whilst it is hard to quantify an increase of a score of 1 for the 'healthy diet' infant feeding pattern, a healthier diet did appear to have an effect size (RR 0.577) worthy of action. Consequently, public health policy to reduce allergy incidence would be similar to that for general health in infancy and young childhood and fits with the core requirements of the Healthy Child Programme (HCP) (274). The problem with this is that compared to previous messages regarding food avoidance for allergy prevention, it does not seem very pro-active and so may not be readily embraced.

The second study finding that introducing solids into the infant diet before 17 weeks of age appears to be associated with allergy development is also in adherence with current UK population infant feeding recommendations. Consequently existing feeding recommendations that advocate delaying the introduction of solids into the diet would appear to be have a small protective effect (RR 0.847).

The third finding regarding concurrent breastfeeding whilst introducing allergenic food into the diet does not fit with the recommendations so readily. Currently, mothers are advised to avoid introducing any solids into the infants' diet before 26 weeks of age and to aim to exclusively breastfeed up to this point. They are also strongly advised not to introduce any allergenic foods before 26 weeks (172). If this advice is adhered to, only infants whose mothers choose to breastfeed beyond 26 weeks will have the opportunity of first encountering allergenic foods whilst still being breastfed. Of this study cohort, only 3% of infants fell into this category. To increase concurrent breastfeeding rates, mothers would need to breastfeed their infants for longer or feeding advice would need to be changed to state foods can be introduced from 17 weeks of age. This would mean the UK no longer supporting the WHO infant feeding recommendation to breastfeed exclusively for 26 weeks. The WHO advice is designed to reduce infant mortality and morbidity rates (23), particularly for infants born in developing countries. Whilst it is important that developed countries support infant feeding recommendations directed at low and middle income countries, a one size fits all recommendation is unlikely to deliver reduced disease risk to all populations. Therefore, the public health question to ask in each context is does the benefit of exclusive breastfeeding outweigh any potential increased risk of developing food allergy? To establish the big picture effect of changing the recommendation for solids to be introduced before 26 weeks of age, risk calculations need to be made to establish how many infants would have an increased risk of disease (particularly gastro-intestinal disease) compared to the reduced numbers of infants developing food allergy. As proposed by Fewtrell et al (11), there is an urgency that these issues are addressed by the UK DoH under the guidance of SACN to see if a position where

developed countries can support WHO infant recommendations to the full whilst still issuing recommendations appropriate for their own nation's infants is possible. In the meantime, however, encouraging mothers to continue to breastfeed beyond 26 weeks so that there is an overlap between breastfeeding and the introduction of allergenic foods seems the sensible action.

In the longer term, further research needs to be carried out to add to the findings of this study. Two main questions about the timing of solid introduction remain: is there a time by which solids need to be introduced into the diet; and is the timing of concurrent breastfeeding important? There is currently a Food Standards Agency funded project (The EAT Study) looking at the issue of concurrent breastfeeding with allergenic foods which should provide data that can go towards answering these questions. However, it is of note that the study protocol only encouraged breastfeeding to 26 weeks, so it may not have adequate data to establish if the protective effect of concurrent breastfeeding goes beyond 6 months of age. If these two questions can be answered, it would establish whether the infant feeding public health message should emphasise 'overlap' or duration of breastfeeding. Additionally new cohort studies using the findings and unique methodologies from this study to inform its design (including its sample size calculations) can provide data to fill the current knowledge gaps regarding the ideal timing and nature of solid introduction.

These recommended studies could also investigate the observations from this research concerning maternal diet which cannot be answered just by further analysis of all the EuroPrevall data. The studies would ideally be undertaken analyzing detailed prospective dietary intake data combined with immunological factors of maternal and infant serum, as well as those for breast milk, so that possible mechanisms of observed effects can be established.

In conclusion, the data from this study have provided some unique findings that can support current infant feeding and allergy prevention recommendations and can inform further research in the field.

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Appendix 1 Tables detailing main papers reviewed

The levels of evidence stated in the following tables are from the Centre of Evidence-based Medicine, Oxford.

The levels for therapy/prevention/etiology/harm were used and are as follows;

1a: Systematic reviews (with homogeneity) of randomised controlled trials
1b: Individual randomised controlled trials (with narrow confidence intervals)
1c: All or none randomised controlled trials
2a: Systematic reviews (with homogeneity) of cohort studies
2b: Individual cohort study or low quality randomised controlled trials)e.g <80% follow-up)
2c: “Outcomes” research; ecological studies
3a: Systematic review (with homogeneity) of case-control studies
3b: Individual case-control study
4: Case-series (and poor quality cohort and case control studies)
5. Expert opinion without explicit critical appraisal, or based on physiology, bench research or “first principles”. (275)

Table A1.1 Details of papers detailing maternal nutrition and allergy development.

Study	Reference Number	Type Of Paper	Study Design	n	Population	Outcome Measure	Result	Statement Of Evidence†
Calvani et al, 2006	82	Original Research	Retrospective cohort study. Maternal dietary intake of fish, butter and margarine collected retrospectively by questionnaire	988	Children (mostly allergic; mean age 5 years)	Infant sensitisation (by SPT)	Maternal fish oil intake protective in non-atopic mothers	3b/4
Chatzi et al, 2008	113	Original Research	Prospective birth Cohort Study. Maternal diet data collected via ffq and coded for adherence to Mediterranean diet	412	Mother and Infant pairs	Parent reported wheeze and sensitisation (by SPT) at 6.5 years.	Maternal Mediterranean diet protective for persistent wheeze, atopic wheeze and sensitisation	2b
Dunstan et al, 2003	84	Original Research	Randomised control trial (RCT) of n-3 supplementation during pregnancy	83	Mother and infant pairs (mother atopic)	Cord blood T-cell responses and physician diagnosed, asthma,	Reduced cytokine responses to all allergens in intervention group. No (real)	1c

Study	Reference Number	Type Of Paper	Study Design	n	Population	Outcome Measure	Result	Statement Of Evidence†
						eczema and sensitisation by SPT at 1 year	difference in any clinical outcome	
Erkkola et al, 2009	100	Original Research	Prospective birth cohort. Retrospective ffq for maternal diet in 8th month of pregnancy	1669	Mother and infant pairs	Parental report of physician diagnosed Asthma, eczema and allergic rhinitis at 5 years	Maternal vitamin D intake inversely related to asthma and AR risk	2b
Falth-Magnusson et al, 1987	116	Original Research	RCT with maternal avoidance of milk and egg from 28 week gestation to delivery. Control mothers consumed normal diet	212	Mother and infant pairs (Infants high risk of atopy)	SPT, SpIgE and clinical manifestation of allergic disease at 18 months of age	No difference between the two experimental groups	1c
Furuhjelm et	85	Original	RCT of n-3	145	Mother and infant	Parent	Maternal	1c

Study	Reference Number	Type Of Paper	Study Design	n	Population	Outcome Measure	Result	Statement Of Evidence†
al, 2009		Research	supplementation during pregnancy (and early lactation). Dietary intake assessed by 3 day food diary at start of supplementation		pairs (Infants high risk of atopy)	reported physician diagnosed asthma, eczema and allergic disease, sensitisation at 12 months of age	supplementation of n-3 fish oils resulted in reduced IgE mediated eczema and food allergy	
Gale et al, 2008	98	Original Research	Prospective birth cohort. Maternal 25(OH) Vit-D concentrations taken late pregnancy	440 /178	Mother and infant pairs	Researcher diagnosed eczema at 9 months and parent reported asthma at 9 years	Higher 25(OH) Vit D concentrations associated with increased risk of eczema at 9 months and asthma at 9 years	2b
Haberg et al, 2009	109	Original Research	Prospective birth cohort. Retrospective	32077	Mother and Infant pairs	Parent reported	Supplementation led to slightly	2c

Study	Reference Number	Type Of Paper	Study Design	n	Population	Outcome Measure	Result	Statement Of Evidence†
			questionnaire data on folate supplementation			asthma at 18 months	increased risk of parent reported wheeze	
Herrmann et al, 1996	117	Original Research	RCT. 3 groups; Maternal avoidance of egg and milk during last trimester of pregnancy and 3 month exclusive breastfeeding, maternal avoidance of egg and milk 3 month exclusive breastfeeding and no maternal avoidance	150	Mother and infant pairs (mother family history of atopy)	Physician diagnosed atopic dermatitis and sensitisation (IgE) at 6 and 12 months	No difference between the three experimental groups	1c
Kremmyda et al, 2011	83	Systematic Review	Systematic review into association between early exposure to n-3 fatty acids and atopy risk	20 Studies	Studies of differing methodologies	Differing atopic outcomes	No clear association found. Further research called for	3a

Study	Reference Number	Type Of Paper	Study Design	n	Population	Outcome Measure	Result	Statement Of Evidence†
Lange et al, 2010	114	Original Research	Prospective birth cohort. Maternal diet assessed by ffq , data from which used in PCA analysis of dietary patterns	1376	Mother and infant pairs	Parent reported recurrent infant wheeze, physician diagnosed asthma and eczema at 3 years	No association between dietary patterns and outcomes	2b
Miyake et al, 2010	101	Original Research	Prospective birth cohort with retrospective diet history questionnaire	763	Mother and Infant pairs	Parent reported infant (<24 months) wheeze and eczema	Higher Vitamin D intake associated with decreased risk of infant wheeze and infant eczema	2b
Sausenthaler et al, 2007	80	Original Research	Prospective birth cohort. Maternal intake for last 4 weeks pregnancy by ffq	2641	Mother and infant pairs	Parent reported physician diagnosed eczema and	Maternal intake of margarine, vegetable oils and some allergenic fruit	2b

Study	Reference Number	Type Of Paper	Study Design	n	Population	Outcome Measure	Result	Statement Of Evidence†
						allergic sensitisation (SpIgE) at 2 years of age	and vegetables positively associated with increased risk of eczema and sensitisation. Maternal intake of fish negatively associated	
Vance et al, 2004	118	Original Research	RCT with maternal avoidance of egg during pregnancy (>18 weeks gestation) and lactation	229	Mother and infant pairs (Infants high risk of atopy)	Physician diagnosed eczema, asthma and sensitisation (SPT and SpIgE) at 6 months of age	No difference between the two experimental groups	1c

†Statement of evidence from Centre of Evidence-based Medicine, Oxford (275).

Table A1.2 Papers detailing breastfeeding and allergy development.

Study	Reference Number	Type Of Paper	Study Design	n	Population	Outcome Measure	Result	Statement Of Evidence†
Gdalvich et al, 2001	127	Meta-analysis	Meta-analysis into association between exclusive breast-feeding during the first 3 months and atopic dermatitis.	18 prospective studies	-	Development of Atopic Dermatitis	Exclusive breast-feeding during the first 3 months of life is associated with lower incidence rates of atopic dermatitis in children with a family history of atopy.	2a
Gdalvich et al,	128	Systematic review	Systematic review into association between exclusive breast-feeding during the first 3 months and asthma.	18 prospective studies	-	Development of Asthma	Exclusive breast-feeding during the first months after birth associated with lower asthma rates during childhood.	2a

Study	Reference Number	Type Of Paper	Study Design	n	Population	Outcome Measure	Result	Statement Of Evidence†
Halpern et al, 1973	131	Original research	Prospective birth cohort of three feeding groups; breast milk, soy formula, standard infant formula	1753	General population	Allergy by age 7	12% infants challenge diagnosed with food allergy. No difference in how these infants were fed	2c
Kramer and Kakuma, 2004	129	Systematic review	Systematic review into association between exclusive breastfeeding for 6 months vs. exclusive breastfeeding for 3-4 months.	20 studies	Different study methodologies	Weight and length gain, weight-for-age and length-for-age z-scores, head circumference, iron status, gastrointestinal and respiratory infectious morbidity, atopic eczema, asthma, and neuromotor	No evidence to suggest infants who continue to be exclusively breastfed for 6 months show deficits in weight or length gain. Exclusive breastfeeding for 6 months or more did not	2a

Study	Reference Number	Type Of Paper	Study Design	n	Population	Outcome Measure	Result	Statement Of Evidence†
						development.	demonstrate a significant reduction in risk of atopic eczema, asthma, or other atopic outcomes.	
Kramer et al, 2007	130	Original Research	Cluster randomised trial of promotion of exclusivity and duration of breastfeeding	13,889	Mother and infant pairs	Physician diagnosed asthma and allergy and sensitisation (by SPT) at 6.5 years	No difference between the groups (ie longer duration and duration of exclusivity did not appear to confirm benefit)	1c
Lauberau et al 2004	141	Original Research	Prospective birth cohort	3903	General population	Physician diagnosed Atopic	No association between	2b

Study	Reference Number	Type Of Paper	Study Design	n	Population	Outcome Measure	Result	Statement Of Evidence†
			study. Diet data collected by retrospective questionnaire			Dermatitis at 3 years	exclusive breastfeeding and allergy outcome. For high risk infants exclusive breastfeeding was associated with lower risk of AD compared with feeding with standard infant formula	
Mattheson et al, 2007	140	Original Research	Prospective birth cohort study. Feeding data collected by retrospective questionnaire	5659	General population	Parent/participant reported asthma.	No real effect seen for exclusive breastfeeding for 3 months at 7 years but	2b

Study	Reference Number	Type Of Paper	Study Design	n	Population	Outcome Measure	Result	Statement Of Evidence†
							in high risk infant prolonged breastfeeding associated with increased risk at 14 and 44 years	
Saarinen et al, 1979	135	Original Research	Prospective birth cohort study. Feeding regimes 'strictly monitored'. 3 groups, never/ hardly breastfed, 1-6 months breastfed and >6 months breastfed	235	General population	Physician diagnosed asthma, eczema and food allergy and SpIgE at 1 year	Prolonged breastfeeding resulted in lower of severe/obvious atopic disease	2b

Study	Reference Number	Type Of Paper	Study Design	n	Population	Outcome Measure	Result	Statement Of Evidence†
Saarinen et al, 1995	136	Original Research	Prospective birth cohort study. Feeding regimes 'strictly monitored'. 3 groups, never/ hardly breastfed, 1-6 months breastfed and >6 months breastfed	235 (1 year), 177 (3 years), 153 (5 years), 135 (10 years), 150 (17 years)	General population	Physician diagnosed asthma, eczema and food allergy and SpIgE at 1, 3, 5, 10 and 17 years	Prevalence of allergy (all outcomes) highest in group who were never/hardly ever breastfed	2b

†Statement of evidence from Centre of Evidence-based Medicine, Oxford (275).

Table A1.3 Papers detailing infant formula and allergy development

Study	Reference Number	Type Of Paper	Study Design	n	Population	Outcome Measure	Result	Statement Of Evidence†
Bhatia and Greer, 2008	146	Report	-	-	-	-	Few indications for use of soy formula….should not be recommended for use in allergy prevention	5
Mallet and Henocq, 1992	153	Original Research	RCT of hydrolysed formula compared to standard infant formula (fed until 4 months of age)	177	High risk infants	Eczema, Asthma and sensitisation (by SpIgE) at 1, 2 and 4 years of age	No difference between groups at 1 years. Significantly less eczema in hydrolysed formula group at 2 and 4 years	1c
Oldaeus et al, 1997	154	Original Research	RCT of extensively and partially hydrolysed formula compared to standard infant formula	155	High risk infants	Clinical examination and SPT at 6, 9, 12 and 18 months. DBPCFC confirmed food allergy .	Significantly less allergic manifestations during the first 18 months for the extensively hydrolysed group (compared to standard formula)	1c
Osborn and Sinn, 2009	149	Cochrane review	Cochrane review of RCT studies	3 studies	-	Clinical symptoms of allergy	Feeding with soy formula cannot be recommended for prevention of allergy or food intolerance	1a

Study	Reference Number	Type Of Paper	Study Design	n	Population	Outcome Measure	Result	Statement Of Evidence†
Osborn and Sinn, 2009	158	Cochrane review	Cochrane review of RCT studies	15 studies	-	Clinical symptoms of allergy	No evidence to support feeding with a hydrolysed formula compared with exclusive breastfeeding. Limited evidence that prolonged feeding with a hydrolysed compared to standard infant formula reduces risk in high risk infants	1a
Vandenplas et al, 1992	152	Original Research	RCT of whey hydrolysate compared to standard infant formula	67	High risk infants	Symptoms of 'allergic manifestations' including eczema and GI symptoms at 6 and 12 months	Whey hydrolysate decreased the incidence of atopic disease up to the age of 12 months.	1c
Vandenplas et al, 1995	151	Original Research	RCT of partial hydrolysate compared to standard infant formula	58	High risk infants	Symptoms of 'allergic manifestations' including eczema and GI symptoms at 6, 12 36 and 60 months	Significant difference between groups at 6 months. Difference at 12 and 60 months significantly different if cumulative manifestations used. If GI symptoms removed	1c

Study	Reference Number	Type Of Paper	Study Design	n	Population	Outcome Measure	Result	Statement Of Evidence†
							only significant difference at 6 months.	
Von Berg et al, 2003	249	Original Research	RCT. Four infant formula groups; extensively hydrolysed casein, extensively hydrolysed whey, partially hydrolysed whey and standard infant formula	2252 (945 per protocol at 1 year)	High risk infants	Study clinician diagnosed allergic disease (Atopic Dermatitis, GI food allergy, Allergic urticaria or any combination of these factors at 1 year of age.	Allergic manifestation significantly reduced in infants receiving extensively hydrolysed casein formula compared to standard formula. AD significantly reduced in infants receiving extensively hydrolysed casein and partially hydrolysed whey formula	1c
Von Berg, 2008	156	Original Research	RCT. Four infant formula groups; extensively hydrolysed casein, extensively hydrolysed whey, partially hydrolysed whey and standard infant formula	2252 (895 per protocol at 6 years)	High risk infants	Parental reported physician diagnosed allergic disease (Atopic Dermatitis, GI food allergy, Allergic urticaria or any combination of these factors at	In per protocol analysis, all study formula significantly reduced allergic disease and AD compared to standard formula. In intention to treat analysis, the extensively hydrolysed whey formula did not	1c

Study	Reference Number	Type Of Paper	Study Design	n	Population	Outcome Measure	Result	Statement Of Evidence†
						1 year of age.	significantly reduce any outcome	
Zeiger et al, 1989	12	Original Research	RCT of food allergen avoidance (including hydrolysed formula use) compared to standard feeding practices	288	High risk infants	Food allergy, Atopic Dermatitis, Allergic Rhinitis, Asthma, any atopic disease and sensitisation at 12 and 24 months (by SPT)	Intervention group had significantly lower atopic dermatitis, urticaria and/or gastrointestinal disease at 12 months and any positive food skin test at 24 months	1c
Zeiger and Heller, 1995	155	Original Research	RCT of food allergen avoidance (including hydrolysed formula use) compared to standard feeding practices	288 (165 at 7 years)	High risk infants	Food allergy, Atopic Dermatitis, Allergic Rhinitis, Asthma, any atopic disease and sensitisation at 7 years	No difference between the groups for any markers	1c

†Statement of evidence from Centre of Evidence-based Medicine, Oxford (275).

Table A1.4 Papers detailing solid introduction and allergy development.

Study	Reference Number	Type Of Paper	Study Design	n	Population	Outcome Measure	Result	Statement Of Evidence†
Alm et al, 2009	192	Original Research	Prospective Birth Cohort. Dietary data collected retrospectively by ffq	5605	General Population	Parental report of eczema and physician diagnosed eczema	Familial eczema increased risk of infant eczema whilst fish consumption before 9 months and having a bird in the house decreased it	2b
Fergusson et al, 1981	175	Original Research	Prospective Birth cohort. Diet data collected from prospective clinical records	1156	General Population	Parent reported eczema	At 2 years cumulative eczema rates increased in direct proportion with the number of different foods introduced before 4 months of age	2b

Study	Reference Number	Type Of Paper	Study Design	n	Population	Outcome Measure	Result	Statement Of Evidence†
Fergusson et al, 1994	1994	Original Research	Prospective Birth cohort. Diet data collected from prospective clinical records	1067	General population	Parent reported childhood eczema up to age 10 years	Increased risk (1.5) of childhood eczema if any solids were introduced during 1st four months of age	2b
Filipiak et al, 2007	183	Original Research	Prospective birth cohort consisting of 2 study groups. Diet data collected retrospectively by questionnaire	4753	General population (separated into high risk and low risk infants)	Cumulative parent reported physician diagnosed eczema at age 4 years	No evidence of protective effect of delaying solid introduction on eczema. Significantly increased risk of eczema with egg avoidance in first year	2b
Kajosaari and Saarinen,	177	Original research	Prospective Birth Cohort. Diet data	135	High risk infants	Parental reported eczema and food	Infants who received no	2b

Study	Reference Number	Type Of Paper	Study Design	n	Population	Outcome Measure	Result	Statement Of Evidence†
1983			collected by retrospective questionnaire			allergy at 1 year	solids before 6 months had less eczema and food allergy	
Kajosaari, 1991	178	Original Research	Prospective Birth Cohort. Diet data collected by retrospective questionnaire	135	High risk infants	Parental reported eczema and food allergy at 5 years	No difference between the groups	2b
Koplin et al, 2010	191	Original Research	Cross-sectional study. Diet data collected by retrospective questionnaire	2589	General population	Physician diagnosed eczema (included by food challenge)	Introduction of egg after 4-6 months was associated with a higher risk of egg allergy	2c
Kull et al, 2006	193	Original Research	Prospective Birth Cohort. Diet data collected by retrospective questionnaire	4089	General Population	Parental report of eczema, asthma, rhinitis at 4 years	Regular fish consumption in first year associated with reduced risk of	2b

Study	Reference Number	Type Of Paper	Study Design	n	Population	Outcome Measure	Result	Statement Of Evidence†
							allergic disease	
Poole et al, 2006	184	Original Research	Prospective Birth Cohort. Diet data collected retrospectively by interview	1612	General Population	Parent reported wheat allergy up to age 4 years	Cereal exposure after 6 months of age associated with increased risk of wheat allergy	2b
Snijders et al, 2008	182	Original Research	Prospective Birth Cohort. Diet data collected by retrospective questionnaire	2558	General Population	Parent reported eczema, physician diagnosed AD and sensitisation (SpIgE) at 2 years	Delayed introduction of Cows milk associated with higher risk for eczema. Also delayed introduction of other solids led to increased risk of allergic disease	2b

Study	Reference Number	Type Of Paper	Study Design	n	Population	Outcome Measure	Result	Statement Of Evidence†
Tarini et al, 2006	179	Systematic review	To assess evidence that early solid feeding (before 4 months of age) increases risk of allergic disease	13 studies	-	Allergic disease	Early solid feeding may increase risk of eczema but no evidence to show early solid feeding is associated with development of other allergic disease. However, more studies required	2a
Zutavern et al, 2004	180	Original Research	Prospective Birth Cohort. Diet data collected by retrospective questionnaire	642	General Population	Physician diagnosed eczema, sensitisation (SPT) and wheeze at 5.5 years	No evidence of protective effect of late introduction of solids for any outcome measure	2b

Study	Reference Number	Type Of Paper	Study Design	n	Population	Outcome Measure	Result	Statement Of Evidence†
Zutavern et al, 2006	248	Original Research	Prospective Birth Cohort. Diet data collected by retrospective questionnaire	2612	General Population	Parental reported eczema, asthma, allergic rhinitis, food allergy and sensitisation at 2 years	No evidence to support introduction of solids beyond 6 months of age protective against the development of allergy.	2b
Zutavern et al, 2008	181	Original Research	Prospective Birth Cohort. Diet data collected by retrospective questionnaire	2073	General Population	Parental reported eczema, asthma, allergic rhinitis, food allergy and sensitisation at 6 years	No evidence to support delaying introduction of solids into the diet beyond '4 or 6 months' for the prevention of any allergic outcome	2b

†Statement of evidence from Centre of Evidence-based Medicine, Oxford (275).

Appendix 2 Variables used in the initial coding of the infant food diaries

The following variables were used in the initial coding of the infant food diaries (see section 3.2.1.2)

Breast milk	Pizza	Mango
Casein based infant formula	Potato Products	Melon
Follow-on infant formula	Ready Meals	Nectarine
Soya based infant formula	Cook-in-sauce	Oranges/citrus
Hydrolysed infant formula	Avocado	Passion fruit
Whey based infant formula	Beets	Peaches
Growing up milk	Broccoli	Pears
Cows' milk	Butternut Squash	Pineapple
Cows' milk as an ingredient	Cabbage/Spinach	Raspberry
Cheese	Carrots	Strawberry
Other mammalian milk	Cauliflower	Any fruit (raw/unblended)
Yogurt/Fromage frais	Celery	Bacon
(Hens) egg	Courgettes	Beef
(Hens) egg as an ingredient	Garlic	Ham
Hummus	Green Beans	Lamb
Peanuts	Leeks	Pork
Seeds	Mushroom	Poultry
Sesame	Onions	Sausages
Tree Nuts	Parsnips	Jam
Crustaceans	Peas	Marmite
Oily fish	Peppers	Fried Foods
White fish	Potatoes	High fibre foods
Bread	Swede	Chocolate
Corn	Sweet Potato	Sweeties
Oats	Sweetcorn	Crisps
Pasta	Tinned/Baked Beans	Dried Fruit
Rice	Tomatoes	Toddler Packet Snacks
Rye	Chickpeas	Fruit Juice
Wheat	Lentils	Fruit Squash
Biscuits/Cake	Apples	Low fat/diet products
Baby Cereal	Apricot	Prebiotics
Commercially prepared savoury baby food	Banana	Probiotics
Commercially prepared sweet baby food	Blackcurrant	Mustard
Commercially prepared savoury baby food (at least 7 times in week)	Blueberry	Soya
Commercially prepared sweet baby food (at least 7 times in week)	Grapes	
Fast food	Kiwi	

Appendix 3 Copy of Ethical Approval Letter for PIFA Study

NHS

North & Mid Hampshire Local Research Ethics Committee

CPW/sks

1ST Floor, Regents Park Surgery
Park Street, Shirley
Southampton
SO16 4RJ

23 September 2005

Doctor Graham Roberts
Clinical Senior Lecturer Paediatric Allergy & Respiratory Medicine
University of Southampton
Child Health, Level F South block (803)
Southampton General Hospital
SOUTHAMPTON
SO16 6YD

Tel: 023 8036 2563
023 8036 3462
Fax: 023 8036 4110

clair.wright@nhs.net
General Enquiries: sharon.atwill@nhs.net
Application Submission: submissions@gp-j82203.nhs.uk

Dear Doctor Roberts

Full title of study: **The Prevalence of food allergy and weaning practises in a birth cohort of UK infants.**

REC reference number: **05/Q1703/34**

Thank you for your letter of 18 August 2005, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information was considered at the meeting of the Sub-Committee of the REC held on 23 September 2005. A list of the members who were present at the meeting is attached.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised.

Conditions of approval

The favourable opinion is given provided that you comply with the conditions set out in the attached document. You are advised to study the conditions carefully.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Application	4.1	14 June 2005
Investigator CV Chief Investigator CV		01 April 2005
Investigator CV Principal Investigator CV		
Protocol		14 June 2005
Covering Letter		13 June 2005
Questionnaire 30 month questionnaire		
Questionnaire 24 month questionnaire		
Questionnaire 12 month questionnaire		
Questionnaire Physician questionnaire for symptomatic visit		
Questionnaire Visit questionnaire (symptomatic and control infant/child)		

An advisory committee to Hampshire and Isle of Wight Strategic Health Authority

Questionnaire At birth		
Questionnaire Intake, at birth	2 (amended)	08 August 2005
Questionnaire 12 month	2 (amended)	08 August 2005
Questionnaire 24 month	2 (amended)	08 August 2005
Questionnaire 30 month	2 (amended)	08 August 2005
Questionnaire Visit	2 (amended)	08 August 2005
Sample Diary/Patient Card	1	14 June 2005
Advertisement Poster/flyer	1	14 June 2005
Letter of invitation to participant	1	24 May 2005
GP/Consultant Information Sheets	1	24 May 2005
GP/Consultant Information Sheets	2 (amended)	05 August 2005
Participant Information Sheet	2 (amended)	14 August 2005
Participant Information Sheet	1	24 May 2005
Participant Consent Form	1	14 June 2005
Participant Consent Form	2 (amended)	14 August 2005
Response to Request for Further Information		16 August 2005
Evaluation summary report for integrated project		
PIFA study protocol	2 (amended)	14 August 2005
Physical examination	2 (amended)	08 August 2005
Food Diary Record	2 (amended)	
Food Allergy poster	Marked version	
Study Introduction Sheet	2 (amended)	
Letter from fund provider		06 May 2005

Research governance approval

The study should not commence at any NHS site until the local Principal Investigator has obtained final research governance approval from the R&D Department for the relevant NHS care organisation.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

05/Q1703/34	Please quote this number on all correspondence

With the Committee's best wishes for the success of this project

Yours sincerely

Jane Ogden-Swift
Chair

Email: GM.E.hio-au.SWHRECA@nhs.net

Enclosures: *List of names and professions of members who were present at the meeting and those who submitted written comments.*

Standard approval conditions,
Site approval form

Copy to: Southampton University Hospitals Trust
R + D Office
Trust Management Offices, Mailpoint 18
Tremona road, Southampton
SO16 6YD

Appendix 4 Researcher participation in main PIFA study methodology

KG was directly involved in the following elements of the PIFA study methodology:

Design of the EuroPrevall protocol and the application of the PIFA study to ethics.

Recruitment of study participants (over 850 of the final 1170).

Sending diet diaries out to participants.

Database entry of the baseline questionnaire data.

Assessment of diet diaries on receipt into study office to ensure adequacy of data.

Triage of suspected food allergic infants (done solely by KG).

Delivery of dietary elimination information to parents (done solely by KG).

Preparation of DBPCFC materials.

Decoding of DBPCFC (done solely by KG) for diagnosis of food allergic infants.

Dietary follow-up of food allergic infants (done solely by KG).

Inputting of quantitative dietary intake data into nutritional analysis package for symptomatic and control infants.

Coding and data entry of all food diaries received (n=910) (done solely by KG).

Appendix 5 Questions regarding Maternal diet taken from EuroPrevall Baseline questionnaire

Prevalence of Infant Food Allergy
Ethical Approval 05/Q1703/34

C. Questions about your pregnancy

From the list below please indicate whether you ate foods in each group AND whether you ate the same amount, more or less, or if you avoided it all together. Please tick the relevant boxes.

	Did you eat this food before you were pregnant		Did you eat this food whilst you were pregnant?		If you did eat this food during pregnancy…..			
					….did you eat the same amount	….did you eat more?	….did you eat less?	….did you avoid the food altogether?
Milk and diary	Yes	No	Yes	No				
Soy Products (eg milk, soya)	Yes	No	Yes	No				
Eggs	Yes	No	Yes	No				
Peanuts	Yes	No	Yes	No				
Other nuts	Yes	No	Yes	No				
Seeds (eg sesame, sunflower)	Yes	No	Yes	No				
Fish	Yes	No	Yes	No				
Shellfish	Yes	No	Yes	No				
Cereals and cereal products (eg pasta, rice, bread)	Yes	No	Yes	No				
Vegetables	Yes	No	Yes	No				
Legumes	Yes	No	Yes	No				
Fruit	Yes	No	Yes	No				
Meat and Meat products	Yes	No	Yes	No				

Coffee and tea (not decaf.)	Yes	No	Yes	No				
Alcohol	Yes	No	Yes	No				
Confectionaries	Yes	No	Yes	No				
Fish liver oil	Yes	No	Yes	No				
Probiotics	Yes	No	Yes	No				

EuroPrevall Baseline Questionnaire

Form 2

Questions for Mother

06.01.06

Appendix 6 Infant Food Diary Record

PIFA

Prevalence of Infant Food Allergy

Instructions for completion

Simply write down anything your infant eats or drinks e.g. a breastfeed, potato and carrot puree etc.

Please give details of what is given (eg ingredients in a homemade dish or brand and type of commercial food).

On week 4 of each month (indicated by the blue sheet) please give more details of amount taken
eg, 4fl oz, formula milk (stating brand and type), 3 teaspoons potato and parsnip puree etc.

Try to write down when things are taken so foods aren't forgotten.

For most days it should take only a minute or two to complete, for the 4th week in every month we would like a little more detail but we DO NOT require you to weigh or measure foods. Household measures or packet size is plenty of information.

Feel free to contact Kate Grimshaw the study's research dietitian if you have any questions. Her number is 023 8079 4230.

Thank you for completing these diaries.
LREC number 05/Q1703/34

Version 2

05.08.05

Study Number 1800 Diary Wk 1 / Child's age in wks =

	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Morning							
Afternoon							
Evening							
Night							

Study Number 1800 Diary Wk 2 / Child's age in wks =

	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Morning							
Afternoon							
Evening							
Night							

Study Number 1800 Diary Wk 3 / Child's age in wks =

	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Morning							
Afternoon							
Evening							
Night							

Study Number 1800 Diary Wk 4 / Child's age in wks =

	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Morning							
Afternoon							
Evening							
Night							

Appendix 7 Comparison of mean values and standards deviations from dietary analysis of 10 complete sets of diaries by the separate researchers

In all cases n=30	Energy (kcal)		Protein (g)		Fat (g)		Iron (mg)		Zinc (mg)		Selenium (ug)		Vitamin A (ug)		Vitamin C (mg)		Vitamin E (mg)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Month 1	504.3	38.9	10.3	0.82	28.8	2.97	0.8	0.08	2.3	0.20	10.8	0.92	557.4	24.61	38.2	3.96	3.3	0.40
Month 2	559.4	32.8	10.9	0.65	32.4	2.70	0.62	0.07	2.96	0.35	9.0	1.04	526.6	35.55	42.1	3.83	3.9	1.74
Month 3	520.3	43.26	10.3	1.07	30.2	3.22	0.6	0.02	2.85	0.44	8.36	0.41	501.7	42.6	39.9	4.74	3.3	0.67
Month 4	619.1	54.76	13.5	1.62	34.8	4.34	0.79	0.11	3.2	0.22	10.4	1.68	565.8	32.58	49.3	4.78	4.7	0.36
Month 5	673.1	76.22	14.4	1.48	33.8	4.27	2.19	0.29	3.5	0.51	10.5	1.41	761.3	39.39	58.8	7.67	5.5	0.83
Month 6	765.9	84.32	16.2	0.93	36.3	4.00	6.09	0.58	3.5	0.56	10.7	1.48	729.0	47.00	65.7	5.27	5.19	0.56

In all cases n=30	Energy (kcal)		Protein (g)		Fat (g)		Iron (mg)		Zinc (mg)		Selenium (ug)		Vitamin A (ug)		Vitamin C (mg)		Vitamin E (mg)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Month 7	829.6	129.24	21.3	2.79	24.1	3.22	10.2	1.09	4.67	0.23	12.0	1.84	1831.1	73.64	85.0	6.50	17.9	1.21
Month 8	752.2	68.26	22.5	2.73	29.6	4.44	7.22	1.31	4.5	0.60	13.1	1.80	740.5	93.73	70.0	5.42	5.6	0.89
Month 9	828.7	43.7	24.9	3.04	32.8	5.02	8.07	0.72	4.5	0.53	13.8	2.51	677.1	104.4	78.3	9.50	8.5	1.41
Month 10	877.7	95.9	27.4	4.12	33.6	4.37	6.1	1.15	4.4	0.79	14.6	1.85	713.7	117.66	80.5	8.27	11.7	2.28
Month 11	976.2	113.9	32.0	4.40	38.2	6.16	6.7	0.47	5.1	0.85	15.5	2.66	668.4	108.59	69.8	9.25	10.67	1.32
Month 12	1157.8	157.5	35.9	3.72	45.5	7.54	8.0	1.17	4.9	0.65	17.7	3.60	646.5	129.37	95.4	7.74	20.1	2.00
Month 13	1177.2	127.1	39.6	2.99	48.7	6.48	7.4	0.99	5.5	0.72	19.9	2.80	700.9	107.95	84.5	7.60	23.3	2.92

Appendix 8 Table detailing power of analyses and numbers required for 90% at α level of 5% for differing variables

Variable		Symptomatic (n=41)	Control (n=82)	p value of study results	Odds ratio /Effect size (d) of study results	Powering of study results (%)	Numbers required for 90% power at 5% level
Maternal Asthma (%)		11 (26.8)	11 (13.4)	0.067	0.55†	51	300
Maternal Allergy (%)		22 (53.7)	31 (37.8)	0.105	1.92†	43	356
Maternal multi-vitamin use (%)	During pregnancy	24 (58.5)	47 (57.3)	0.495	1.45†	5	21,058
	During lactation	9 (22.0)	20 (24.4)	0.404	1.09†	5	15,64
Maternal folic acid supplement use (%)	During pregnancy	36 (87.8)	69 (84.1)	0.345	0.89†	9	2,666
	During lactation	7 (17.1)	9 (11.0)	0.320	1.40†	17	1.198
Maternal vitamin D supplement use (%)	During pregnancy	2 (4.9)	1 (1.2)	0.241	7.05†	21	700
	During lactation	3 (9.8)	2 (2.4)	0.444	5.21†	13	1,354
Maternal fish oil supplement use (%)	During pregnancy	3 (9.8)	13 (17.0)	0.235	0.54†	19	862
	During lactation	2 (4.9)	5 (6.1)	0.514	0.82†	2	18,200
Maternal paracetomol use (%)	During pregnancy	29 (80.5)	50 (68.4)	0.352	0.82†	38	410
	During lactation	31 (86.1)	47 (70).1	0.119	2.00†	57	246

Variable		Symptomatic (n=41)	Control (n=82)	p value of study results	Odds ratio /Effect size (d) of study results	Powering of study results (%)	Numbers required for 90% power at 5% level
Maternal NSAID use (%)	During pregnancy	4 (11.4)	7 (9.5)	0.709	2.63†	12	1,834
	During lactation	16 (44.4)	16 (24.4)	0.047	2.69†	50	296
Maternal antacid use (%)	During pregnancy	13 (37.1)	24 (32.9)	0.01	2.07†	66	208
	During lactation	0	2 (3)	n/a	-	-	-
Maternal antibiotic use (%)	During pregnancy	9 (22.0)	19 (23.2)	0.349	0.944†	4	6,0130
	During delivery	7 (17.1)	8 (9.8)	0.177	1.84†	22	862
	After delivery	9 (22.0)	11 (13.4)	0.160	1.89†	28	644
	During lactation	16 (39.0)	18 (22.0)	0.349	2.12†	52	360
'Full' maternal diet (%)	During pregnancy	4 (9.8)	22 (26.8)	0.032	3.0†	19	862
	During lactation	6 (14.6)	18 (22.0)	0.114	0.5†	17	1,102
Breastfeeding initiation rate (%)		38 (92.7)	79 (96.3)	0.333	1.6†	11	2,074
Mean breastfeeding duration (weeks)		19.2	21.14	0.295	0.12§	7	4,226
Mean exclusive breastfeeding duration (weeks)		9.77	9.73	0.933	0.12§	15	2,526
Mean duration of concurrent breastfeeding and any solid food (weeks)		5.37	6.33	0.303	0.1§	13	3,370
Mean duration of concurrent breastfeeding and any cows'		9.84	12.54	0.047	0.22§	30	736

Variable		Symptomatic (n=41)	Control (n=82)	p value of study results	Odds ratio /Effect size (d) of study results	Powering of study results (%)	Numbers required for 90% power at 5% level
milk protein (weeks)							
Use of prebiotic supplemented infant formula (%)		16 (39.0)	50 (61.0)	0.094	0.6†	68	194
Mean age at first introduction of formula (weeks)		7.87	8.31	0.478	0.05§	8	21,220
Mean age at introduction of solids (weeks)		18.68	20.04	0.044	0.34§	54	310
Mean age at introduction of cows' milk ingredient (weeks)		24.43	25.61	0.049	0.15§	18	1,682
Mean age at introduction of Egg as ingredient (weeks)		41.2	36.96	0.067	0.46§	75	174
Mean 'Ongoing' infant diet pattern		-.383	.192	0.002	0.6§	92	102

*† Odds Ration § Effect size (d). All values calculated using 'G*Power version 3.1.3*

Appendix 9 Table detailing power of analyses and numbers required for 90% at α level of 5% for nutrients

Nutrient of interest		Mean daily intake for symptomatic infants (n=41)	Mean daily intake for control infants (n=82)	p value of study results	Effect size of study results (d)	Powering of study results (%)	Numbers required for 90% power at 5% level
Energy							
	Month 1	518.4	512.6	0.6	0.11	12	3,080
	Month 2	573.5	587.5	0.32	0.27	23	486
	Month 3	587.6	608.5	0.22	0.32	29	342
	Month 4	643.1	675.9	0.50	0.30	22	408
	Month 5	688.6	646.2	0.21	0.36	28	278
	Month 6	707.4	708.2	0.98	0.01	5	847,482
	Month 7	812.1	739.6	0.13	0.74	49	68
	Month 8	774.9	788.0	0.8	0.14	8	1,770
	Month 9	862.6	838.3	0.76	0.21	8	788
	Month 10	1047.1	881.0	0.18	1.50	58	18

Nutrient of interest		Mean daily intake for symptomatic infants (n=41)	Mean daily intake for control infants (n=82)	p value of study results	Effect size of study results (d)	Powering of study results (%)	Numbers required for 90% power at 5% level
	Month 11	1045.3	880.8	0.26	1.19	30	28
	Month 12	1041.8	1154.5	0.6	0.60	14	104
	Month 13	1083.9	1085.0	0.99	0.01	5	1,069,710
Protein							
	Month 1	10.1	9.9	0.55	0.15	16	1,704
	Month 2	11.0	11.6	0.12	0.48	48	158
	Month 3	11.3	12.1	0.12	0.52	53	138
	Month 4	12.9	13.8	0.51	0.29	21	426
	Month 5	14.9	13.2	0.15	0.41	33	216
	Month 6	14.5	15.1	0.63	0.17	13	1226
	Month 7	19.6	19.8	0.93	0.03	6	31,208
	Month 8	24.9	20.5	0.29	0.83	34	54
	Month 9	26.9	23.8	0.43	0.63	20	92

Nutrient of interest		Mean daily intake for symptomatic infants (n=41)	Mean daily intake for control infants (n=82)	p value of study results	Effect size of study results (d)	Powering of study results (%)	Numbers required for 90% power at 5% level
	Month 10	34.1	25.3	0.05	1.77	71	14
	Month 11	35.3	27.3	0.03	3.27	90	6
	Month 12	33.2	34.2	0.82	0.25	8	580
	Month 13	37.6	36.7	0.82	0.25	8	562
Fat							
	Month 1	29.9	29.7	0.74	0.07	9	7,504
	Month 2	33.6	33.7	0.90	0.03	6	45,326
	Month 3	34.0	34.6	0.54	0.10	9	3,634
	Month 4	36.4	37.9	0.57	0.24	18	586
	Month 5	36.8	35.2	0.30	0.31	23	374
	Month 6	35.5	35.6	0.98	0.02	6	108,598
	Month 7	36.7	31.6	0.21	0.63	39	94
	Month 8	30.8	34.1	0.36	0.56	21	118

Nutrient of interest		Mean daily intake for symptomatic infants (n=41)	Mean daily intake for control infants (n=82)	p value of study results	Effect size of study results (d)	Powering of study results (%)	Numbers required for 90% power at 5% level
	Month 9	33.9	36.1	0.72	0.22	8	758
	Month 10	46.2	35.2	0.14	1.28	48	24
	Month 11	40.6	36.4	0.61	0.48	12	158
	Month 12	38.5	48.5	0.31	0.91	21	46
	Month 13	42.3	45.6	0.78	0.29	8	434
Iron							
	Month 1	1.4	1.4	0.91	0.00	5	Utc
	Month 2	1.2	2.0	0.15	0.48	48	160
	Month 3	1.7	2.4	0.28	0.36	33	280
	Month 4	2.4	3.5	0.32	0.35	27	292
	Month 5	3.3	3.7	0.63	0.1	10	2,085
	Month 6	3.5	4.7	0.22	0.42	34	208
	Month 7	6.5	6.9	0.78	0.12	8	2,584

Nutrient of interest		Mean daily intake for symptomatic infants (n=41)	Mean daily intake for control infants (n=82)	p value of study results	Effect size of study results (d)	Powering of study results (%)	Numbers required for 90% power at 5% level
	Month 8	6.7	6.9	0.91	0.05	6	13,112
	Month 9	8.0	8.7	0.75	0.21	8	798
	Month 10	6.6	9.4	0.34	1.00	35	38
	Month 11	10.2	7.0	0.10	2.24	66	10
	Month 12	9.5	8.2	0.46	0.69	16	78
	Month 13	8.6	6.8	0.27	0.81	19	56
Zinc							
	Month 1	2.3	2.3	0.94	0.00	5	Utc
	Month 2	2.4	2.9	0.09	0.50	51	146
	Month 3	2.6	3.3	0.06	0.61	66	98
	Month 4	2.6	3.7	0.06	0.59	54	104
	Month 5	3.2	3.6	0.51	0.26	18	598
	Month 6	3.2	4.0	0.14	0.53	46	132

Nutrient of interest		Mean daily intake for symptomatic infants (n=41)	Mean daily intake for control infants (n=82)	p value of study results	Effect size of study results (d)	Powering of study results (%)	Numbers required for 90% power at 5% level
	Month 7	4.8	4.7	0.88	0.07	7	7,644
	Month 8	4.5	4.6	0.86	0.09	7	4,588
	Month 9	4.6	5.4	0.38	0.72	23	72
	Month 10	5.1	5.8	0.67	0.44	14	192
	Month 11	6.2	4.9	0.33	1.18	29	28
	Month 12	5.5	5.7	0.85	0.19	7	1,000
	Month 13	5.9	5.1	0.31	1.03	25	36
Selenium							
	Month 1	8.3	8.5	0.7	0.00	11	4,020
	Month 2	8.9	9.7	0.14	0.46	45	174
	Month 3	9.5	10.2	0.29	0.34	30	316
	Month 4	10.3	11.1	0.44	0.25	18	592
	Month 5	11.4	11.6	0.83	0.06	7	10,342

Nutrient of interest		Mean daily intake for symptomatic infants (n=41)	Mean daily intake for control infants (n=82)	p value of study results	Effect size of study results (d)	Powering of study results (%)	Numbers required for 90% power at 5% level
	Month 6	11.2	11.9	0.44	0.25	18	588
	Month 7	15.6	12.7	0.22	0.56	33	118
	Month 8	13.8	13.9	0.97	0.03	5	46,304
	Month 9	12.6	15.6	0.37	0.68	21	80
	Month 10	14.6	15.4	0.81	0.14	7	1,866
	Month 11	17.6	13.7	0.17	1.61	44	16
	Month 12	18.7	15.0	0.39	0.7	16	74
	Month 13	19.6	19.7	0.97	0.06	6	8,894
Vitamin A							
	Month 1	476.4	483.8	0.61	0.12	13	2,626
	Month 2	521.7	544.3	0.06	0.50	51	144
	Month 3	536.7	568.1	0.11	0.48	48	160
	Month 4	554.9	654.0	0.23	0.51	44	140

Nutrient of interest		Mean daily intake for symptomatic infants (n=41)	Mean daily intake for control infants (n=82)	p value of study results	Effect size of study results (d)	Powering of study results (%)	Numbers required for 90% power at 5% level
	Month 5	666.4	662.4	0.95	0.02	6	87,480
	Month 6	807.4	797.4	0.94	0.02	6	68,844
	Month 7	1210.8	929.6	0.34	0.35	20	294
	Month 8	723.5	1032.4	0.28	0.67	26	82
	Month 9	790.2	881.3	0.68	0.01	5	8,656,256
	Month 10	731.0	818.3	0.69	0.30	10	402
	Month 11	717.9	729.6	0.94	0.08	6	5,288
	Month 12	568.1	747.3	0.09	1.68	46	16
	Month 13	642.8	638.4	0.98	0.03	5	51,584
Vitamin C							
	Month 1	38.3	37.8	0.88	0.03	7	37,292
	Month 2	38.9	45.4	0.15	0.43	42	194
	Month 3	43.7	48.9	0.33	0.10	9	3,640

Nutrient of interest		Mean daily intake for symptomatic infants (n=41)	Mean daily intake for control infants (n=82)	p value of study results	Effect size of study results (d)	Powering of study results (%)	Numbers required for 90% power at 5% level
	Month 4	49.2	61.5	0.24	0.48	40	162
	Month 5	54.0	61.8	0.28	0.36	28	278
	Month 6	63.2	73.2	0.26	0.43	35	200
	Month 7	71.5	85.5	0.18	0.63	40	94
	Month 8	68.3	71.5	0.79	0.17	8	1,266
	Month 9	70.5	73.3	0.88	0.10	6	3,318
	Month 10	81.8	77.1	0.88	0.11	7	2,908
	Month 11	84.0	56.5	0.21	1.11	27	32
	Month 12	108.9	61.1	0.09	1.37	35	22
	Month 13	100.2	52.3	0.01	3.74	95	6
Vitamin E							
	Month 1	3.7	3.6	0.85	0.04	7	19,316
	Month 2	3.8	4.5	0.34	0.26	22	514

Nutrient of interest		Mean daily intake for symptomatic infants (n=41)	Mean daily intake for control infants (n=82)	p value of study results	Effect size of study results (d)	Powering of study results (%)	Numbers required for 90% power at 5% level
	Month 3	4.4	4.8	0.63	0.14	12	1,838
	Month 4	5.1	5.9	0.49	0.22	16	728
	Month 5	5.2	6.3	0.31	0.34	26	310
	Month 6	6.5	6.9	0.70	0.12	10	2,562
	Month 7	11.6	6.9	0.18	0.50	29	148
	Month 8	5.9	5.5	0.68	0.19	9	908
	Month 9	13.6	5.5	0.04	1.39	53	22
	Month 10	16.2	5.9	0.12	0.88	29	48
	Month 11	14.6	4.9	0.19	1.00	24	38
	Month 12	28.2	5.5	0.20	0.95	22	42
	Month 13	32.6	4.4	0.19	0.96	23	42

*All values calculated using 'G*Power version 3.1.3*

Appendix 10 Correlation Matrices for Principal Component Analysis

For all tables, horizontal axis label equates to letter at beginning of the corresponding vertical axis label (e.g ‘a’ to ‘a’, ‘aa to ‘aa’ etc)

Table A10.1 Correlation matrix for Principal Component Analysis for Early infant diet on data from all infants (n=123).

Table A10.2 Correlation matrix for Principal Component Analysis for On-going infant diet on data from all infants (n=123).

Table A10.2 Correlation matrix for final Principal Component Analysis for On-going infant diet on data from all infants (n=123).

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Variable included in the analysis	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	r	s	t	u	v	w	x	y	z	aa	bb	cc	dd	ee
a. Duration of soya and b/milk o/lap	**1.000**	.305	.351	.346	.220	.518	.345	.105	.088	-.099	.102	.309	-.017	.087	.170	.004	.208	.060	.123	.010	.064	.007	.365	.196	.166	.046	.059	.067	.009	.295	.060
b. Duration of fish and b/milk o/lap	.305	**1.000**	.887	.786	.718	.859	.785	.511	.179	.013	-.351	-.023	.195	.161	.034	.124	.040	.103	.020	.208	.034	.086	.539	.028	.132	-.188	-3.77	-.068	.071	.570	.085
c. Duration of wheat and b/milk o/lap	.351	.887	**1.000**	.858	.718	.907	.783	.477	.148	.022	-.138	-.175	.143	.106	-.034	.127	-.004	.103	.020	.208	.034	.086	.539	.028	.132	-.188	-.377	-.068	.071	.570	.085
d. Duration of egg and b/milk o/lap	.346	.786	.858	**1.000**	.638	.830	.675	.429	.223	-.024	-.136	-.026	.203	.172	.039	.117	.070	.179	.037	.246	.059	.173	.523	-.053	-.060	-.051	-.125	-.036	.030	.507	.068
e. Duration of any milk and b/milk o/lap	.220	.718	718	.638	**1.000**	.762	.624	.768	.241	.084	-.024	.113	.267	.114	.014	.234	.048	.247	.186	.244	.108	.072	.046	.055	.121	-.010	-.019	-.052	.099	.129	.012
f. Breastfeeding duration	.518	.859	.907	.830	.762	**1.000**	.822	.577	.308	.004	.036	.235	.272	.198	.109	.236	.120	.277	.118	.314	.103	.157	.618	.218	.253	.051	.019	.097	.054	.619	.112
g. Duration of b/milk and any solid o/lap	.345	.785	.783	.675	.624	.822	**1.000**	.551	.068	-.016	.000	.116	.065	.085	.063	.163	.069	.079	.090	.208	.011	.141	.612	.226	.160	.037	-.031	.025	.084	.608	.144
h. Duration of b/milk and infant formula overlap	.105	.511	.477	.429	.768	.577	.551	**1.000**	.290	-.008	.102	.254	.304	.171	.157	.278	.141	.279	.102	.317	.164	.100	.084	.097	.240	.108	.089	.072	.206	.103	.026
i. Age first solids	.088	.179	.148	.223	.241	.308	.068	.290	**1.000**	-.020	.216	.423	.965	.461	.297	.473	.253	.778	.320	.737	.581	.285	.145	.088	.507	.225	.154	.251	-.077	.109	.176
j. Age first Hummus	-.099	.013	.022	-.024	.084	.004	-.016	-.008	-.020	**1.000**	-.020	-.043	-.030	.057	-.060	.107	-.063	-.005	.083	-.055	-.017	-.064	-.099	.064	-.112	-.030	-.014	-.001	.064	-.017	-.012
k. Age first any fish	.102	-.351	-.138	-.136	-.024	.036	.000	.102	.216	-.020	**1.000**	.437	.188	.113	.168	.271	.168	.300	.239	.247	.169	.171	.110	.396	.272	.649	.905	.364	.020	.044	.105
l. Age first wheat	.309	-.023	-.175	-.026	.113	.235	.116	.254	.423	-.043	.437	**1.000**	.339	.248	.364	.299	.299	.443	.254	.347	.250	.132	.129	.319	.538	.248	.341	.413	.098	.025	.140
m. Age first Baby Cereal	-0.17	0195	.143	.203	.267	.272	.065	.304	.965	-.030	.188	.339	**1.000**	.433	.261	.463	.250	.732	.271	.725	.551	.315	.123	.069	.477	.224	.129	.284	-.070	.100	.142
n. Age first com. savoury baby food	.087	.161	.106	.172	.114	.198	.085	.171	.461	.057	.113	.248	.433	**1.000**	.549	.272	.137	.392	.246	.430	.273	.145	.210	-.011	.378	.051	.082	.104	.119	.089	.321
o. Age first com. sweet baby food	.170	.034	-.034	.039	.014	.109	.063	.157	.297	-.060	.168	.364	.261	.549	**1.000**	.208	.110	.247	.187	.345	.322	.126	.187	-.014	.438	.062	.154	.135	.147	.037	.394
p. Age first yogurt/F Frais	.004	.124	.127	.117	.234	.236	.163	.278	.473	.107	.271	.299	463	.272	.208	**1.000**	.125	.468	.295	.428	.338	.399	.126	.136	.393	.219	.250	.089	.086	.071	.130
q. Age first avocado	.208	.040	-.004	.070	.048	.120	.069	.141	.253	-.063	.168	.299	.250	.137	.110	.125	**1.000**	.333	.182	.298	.221	.189	.121	.079	.321	.145	.148	.268	-.081	.060	.106
r. Age first carrots	.060	.103	.103	.179	.247	.277	.079	.279	.778	-.005	.300	.443	.732	.392	.247	.468	.333	**1.000**	3.42	.819	.664	.366	.112	.140	.436	.305	.242	.266	-.070	.061	.225
s. Age infant first lentils	.123	.020	.029	-.037	.186	.118	.090	.102	.320	.083	.239	.254	.271	.246	.187	.295	.128	.342	**1.000**	.311	.372	.121	-.055	.066	.260	.241	.199	-.023	.093	.009	.112
t. Age first apple	.010	.208	.183	.246	244	.314	.208	.317	.737	-.055	.247	.347	.725	.430	.345	.428	.298	.819	.311	**1.000**	.662	.447	.242	.214	.451	.234	.205	.275	-.039	.186	.405
u. Age infant first had banana	.064	.034	.005	.059	.108	.103	.011	.164	.581	-.017	.169	.250	.551	.273	.322	.338	.221	.664	.372	.662	**1.000**	.316	.048	.156	.421	.240	.132	.078	-.017	.005	.230
v. Age first strawberry	.007	.086	.104	.173	.072	.157	.141	.100	.285	-.064	.171	.132	.315	.145	.126	.399	.189	.366	.121	.447	.316	**1.000**	.151	.132	.217	.120	.168	.132	-.017	.158	.246
w. Age first any cows' milk protein	.365	.539	.572	.523	.046	.618	.612	.084	.145	-.099	.110	.129	.123	.210	.187	.126	.121	.112	.055	.242	.048	.151	**1.000**	.246	.289	.107	.075	.245	-.006	.835	.192
x. Age first egg	.196	.028	.088	-.053	.055	.218	.226	.097	.088	.064	.396	.319	.069	-.011	-.014	.136	.079	.140	.066	.214	.156	.132	.246	**1.000**	.196	.434	.307	.382	.063	.202	.149
y. Age first cow's milk ingredient	.166	.132	.038	.060	.121	.253	.160	.240	.507	-.112	.272	.538	.477	.378	.438	.393	.321	.436	.260	.41	.421	.217	.289	.196	**1.000**	.265	.223	.378	.115	.139	.205
z. Age first oily fish	.046	-.188	-.040	-.051	-.010	.051	.027	.108	.225	-.030	.649	.248	.224	.051	.062	.219	.145	.305	.241	.234	.240	.120	.107	.434	.265	**1.000**	.508	.378	.218	.059	.111
aa. Age first white fish	.059	-.377	-.116	-.125	-.019	.019	-0.31	.089	.154	-.014	.905	.341	.129	.082	.154	.250	.148	.242	.199	.205	.132	.168	.075	.307	.223	.508	**1.000**	.294	-.10	.009	.052
bb. Age first bread	.067	-.068	-.076	-.036	-.052	.097	.025	.072	.251	-.001	.364	.413	.284	.104	.135	.089	.268	.266	.023	.275	.078	.123	.245	.382	.378	.378	.294	**1.000**	.184	.155	.152
cc. Age first b/beans	.009	.071	.031	.030	.099	.054	.084	.206	-.077	.064	.020	.098	-.070	.119	.147	.086	-.081	-.070	.093	-.039	-.017	-.017	-.006	.063	.115	.218	-.010	.184	**1.000**	-.003	1.25
dd. Age first infant formula	.295	.570	.616	.507	.129	.619	.608	.103	.109	-.017	.044	.025	.100	.089	.037	.071	.060	.061	.009	.186	.005	.158	.835	.202	.139	.059	.009	.155	-.003	**1.000**	.135
ee. Age first blueberry	.060	.085	.068	.086	.012	.112	.144	.026	.176	-.012	.105	.140	.142	.321	.394	.130	.106	.225	.112	.405	.230	.246	.192	.149	.205	.111	.052	.152	.125	.135	**1.000**

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Appendix 10 Table A10.2 PCA correlation matrix on-going solids

Variable included in the analysis	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	r	s	t	u	v	w	x	y	z	aa	bb	cc	dd
a. Breastfeeding duration	1.000	.308	.052	.075	-.002	-.041	-.016	.129	-.047	.143	.058	.133	.169	.154	.145	.208	.153	.116	.145	.039	.159	.078	.041	.125	.039	-.042	-.021	.229	.143	.183
b. Age of infant at introduction of solids	.308	1.000	**-.172**	-.169	-.065	-110	-.054	-.119	.050	-.075	-.057	-.168	-.069	-.130	-.065	.032	-.199	.012	.029	-.091	-.052	-.047	-.142	-.144	-.225	-.162	-.143	.054	.017	.100
c. Number of weeks apples were included in the infants diet	.052	-.172	1.000	**.613**	.240	.673	.147	.270	.078	.131	.116	.917	.723	.752	.680	.520	.891	.624	.639	.746	.580	.711	.747	.870	.401	.303	.336	.400	.631	.457
d. Number of weeks infant had commercially prepared savoury baby food	.075	-.169	.613	1.000	**.554**	.718	.317	.072	.187	.265	.200	.653	.623	.624	.473	.264	.647	.379	.480	.560	.350	.520	.635	.698	.307	.144	.306	.012	.378	.272
e. Number of weeks infant had commercially prepared savoury baby food more than once a day	-.002	-.065	.240	.554	1.000	**.451**	.581	.022	.036	.104	.076	.264	.253	.303	.202	.077	.248	.085	.281	.279	.022	.214	.246	.312	.013	.042	.221	-.132	.043	.111
f. Number of weeks infant had commercially prepared sweet baby food	-.041	-.110	.673	.718	.451	1.000	**.320**	.189	.161	.101	.143	.633	.524	.515	.464	.252	.672	.509	.473	.591	.294	.533	.533	.620	.284	.193	281	.097	.399	.348
g. Number of weeks infant had commercially prepared sweet baby food more than once a day	-.016	-.054	.147	.317	.581	.320	1.000	**.034**	.044	.321	.141	.191	.049	.046	.194	.027	.178	.125	.115	.095	.051	.112	.046	.167	.237	.046	.070	-0.89	.098	.289
h. Number of weeks pizza was included in the infants diet	.129	-.119	.270	.072	.022	.189	.034	1.000	**-.027**	.190	.240	.252	.107	.147	.253	.329	.327	.235	.310	.242	.233	.195	.100	.233	.356	.028	.467	.325	.287	.315
i. Number of weeks potato products were included in the infants diet	-.047	.050	.078	.187	.036	.161	.044	-.027	1.000	**.194**	.468	.111	.111	.139	.123	-.005	.085	.056	-.047	.145	.257	-.022	.189	.168	.097	.128	.331	-.033	.047	.095
j. Number of weeks ready meals were included in the infants diet	.143	-.075	.131	.265	.104	.101	.321	.190	.194	1.000	**.460**	.294	.245	.210	.267	-.022	2.91	.325	.158	.247	.277	.134	.288	.258	.426	.171	.230	.067	.164	.277
k. Number of weeks cook-in-sauces were included in the infants diet	.058	-.057	.116	.200	.076	.143	.141	.240	.468	.460	1.000	**.169**	.183	.181	.227	.194	.171	.242	.042	.082	.266	.125	.271	.181	.278	.095	.200	.112	.146	.283
l. Number of weeks carrots were included in the infants diet	.133	-.168	.917	.653	.264	.633	.191	.252	.111	.294	.169	1.000	**.775**	.792	.676	.496	.901	.634	.709	.753	.627	.688	.791	.903	.446	.358	.331	.336	.5936	.468
m. Number of weeks onions were included in the infants diet	.169	-.069	.723	.623	.253	.524	.049	.107	.111	.245	.183	.775	1.000	**.751**	.607	.470	.766	.473	.579	.636	.627	.525	.847	.827	.341	.272	.321	.272	.493	.368
n. Number of weeks potatoes were included in the infants diet	.154	-.130	.752	.624	.303	515	.046	.147	.139	.210	181	.792	.751	1.000	**.547**	.439	.781	.537	.544	.628	.593	.594	.807	.825	.267	.309	.334	.253	.469	.393
o. Number of weeks peas were included in the infants diet	.145	-.065	.680	.473	.202	.464	.194	.253	.123	.267	.227	.676	.607	.547	1.000	**.371**	.670	.475	.489	.532	.492	.566	.624	.643	.238	.111	.362	.241	.505	.431
p. Number of weeks lentils were included in the infants diet	.208	.032	.320	.264	.077	.252	.027	.329	-.005	-.022	.194	.496	.470	.439	.371	1.000	**.455**	.318	.430	.374	.387	.416	.379	.502	.228	.233	.007	.413	.403	.369
q. Number of weeks banana was included in the infants diet	.153	-.199	.891	.647	.248	.672	.178	.327	.085	.291	.171	.901	.766	.781	.670	.455	1.000	**.619**	.669	.770	.615	.635	.767	.839	.454	.312	.281	.330	.604	.486
r. Number of weeks peaches were included in the infants diet	.116	.012	.624	.379	.085	.509	.125	.235	.056	.325	.242	.634	.473	.537	.475	.318	.619	1.000	**.503**	.554	.435	.519	.531	.639	.302	.200	.204	.307	.387	.391
s. Number of weeks raspberries were included in the infants diet	.145	.029	.639	.480	.281	.473	.155	.310	-.047	.158	.042	.709	.579	.544	.489	.430	.669	.503	1.000	**.797**	.519	.610	.603	.622	.353	.270	.196	.327	.478	.504
t. Number of weeks strawberries were included in the infants diet	.039	-.091	.746	.560	.279	.591	.095	.242	.145	.247	.082	.753	.636	.628	.532	.374	.770	.554	.797	1.000	.506	.612	.692	.698	.397	.418	.247	.302	.461	.460
u. Number of weeks oily fish was included in the infants diet	.159	-.052	.580	.350	.022	.294	.051	.233	.257	.277	.266	.627	.627	.593	.492	.387	.615	.435	.519	.506	1.000	.475	.649	.616	.404	.306	.367	.304	.473	.550
v. Number of weeks broccoli was included in the infants diet	.078	-.047	.711	.520	.214	.533	.112	.195	-.022	.134	.125	.688	.525	.594	.566	.416	.635	.519	.610	.612	.475	1.000	.547	.707	.249	.200	.320	.304	.540	.456
w. Number of weeks beef was included in the infants diet	.041	-.142	.747	.635	.246	.553	.046	.100	.189	.288	.271	.791	.847	.807	.624	.379	.767	.531	.603	.692	.649	.547	1.000	.828	.332	.220	.353	.274	.533	.395
x. Number of weeks poultry was included in the infants diet	.125	-.144	.870	.696	.312	.620	.167	.233	.168	.258	.181	.903	.827	.825	.643	.502	.839	.639	.622	.698	.616	.707	.828	1.000	.360	.254	.412	.338	.574	.443
y. Number of weeks Marmite was included in the infants diet	.039	-.225	.401	.307	.013	.284	.237	.356	.097	.426	.278	.446	.3341	.267	.238	.228	.454	.302	.353	.397	.404	.249	3.332	.360	1.000	.406	.212	.249	.463	.476
z. Number of weeks jam was included in the infants diet	-.042	-.162	.303	.144	.042	.193	.046	.028	.128	.171	.095	.358	.275	.309	.111	.233	.312	.200	.270	.418	.306	.200	.220	.254	.406	1.000	-.047	.250	.092	.253
aa. Number of weeks chocolate was included in the infants diet	-.021	-.143	.336	.306	.221	.281	.070	.467	.331	.230	.200	.331	.321	.334	.362	.007	.281	.204	.196	.247	.367	.320	.353	.412	.212	-.047	1.000	.082	.345	.227
bb. Number of weeks dried fruit was included in the infants diet	.229	.054	.400	.012	-.132	.097	-.089	.325	-.033	.067	.112	.336	.272	.253	.241	.413	.330	.307	.327	.302	.304	.304	.274	.338	.249	.25	.082	1.000	.461	.519
cc. Number of weeks toddler packet snacks were included in the infants diet	.143	.017	.631	.378	.043	.399	.098	.287	.047	.164	.146	.596	.493	.469	.505	.403	.604	.387	.478	.461	.473	.540	.533	.574	.463	.092	.345	.461	1.000	.530
dd. Number of weeks fruit (not pureed) was included in the infants diet	.183	.100	.457	.272	.111	.348	.289	.315	.095	.277	.283	.468	.368	.393	.431	.369	.486	.391	.504	.460	.550	.456	.395	.443	.476	.253	.227	.519	.530	1.000

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Variable included in the analysis	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	r	s	t	u	v	w	x	y	z	aa	bb	cc	dd
a. Number of weeks infant had commercially prepared savoury baby food	1.000	.554	**.718**	.317	.195	.187	.265	.200	.176	.520	.653	.613	.142	.285	.698	.281	.307	.144	.012	.378	.272	.350	.119	.302	.443	.490	.502	.417	.232	.124
b. Number of weeks infant had commercially prepared savoury baby food more than once a day	.554	1.000	.451	**.581**	.037	.036	.104	.076	-.108	.214	.264	.240	.081	.447	.312	.056	.013	.042	-.123	.043	.111	.022	-.040	.253	-.001	.199	-.011	.147	-.147	.024
c. Number of weeks infant had commercially prepared sweet baby food	.718	.451	1.000	.320	**.212**	.161	.101	.143	.183	.533	.633	.673	.210	.185	.620	.183	.284	.193	.097	.399	.348	.294	.229	.229	.460	.592	.477	.452	.116	.222
d. Number of weeks infant had commercially prepared sweet baby food more than once a day	.317	.581	.320	1.000	-.072	**.044**	.321	.141	-.035	.112	.191	.147	.112	.484	.167	.229	.237	.046	-.089	.098	.289	.051	.006	.088	.142	.127	.129	.279	-.067	-.006
e. Number of weeks 'fast food' was included in the infants diet	.195	.037	.212	-.072	1.000	.255	**.062**	.107	.113	.231	.296	.327	.123	-.025	.238	.511	.073	-.055	.118	.204	.074	.314	-.018	.302	.303	.199	.235	.210	.037	.226
f. Number of weeks potato products were included in the infants diet	.187	.036	.161	.044	.255	1.000	.194	**.468**	-.093	-.022	.111	.078	-.040	-.054	.168	.304	.097	.128	-.033	.047	.095	.257	.121	.273	.174	-.092	.126	.040	-.029	.213
g. Number of weeks ready meals were included in the infants diet	.265	.104	.101	.321	.062	.194	1.000	.460	**.242**	.134	.294	.131	-.023	.349	.258	.546	.426	.171	.067	.164	.277	.277	.016	.289	.367	.041	.086	.091	.142	.014
h. Number of weeks cook-in-sauces were included in the infants diet	.200	.076	.143	.141	.107	.468	.460	1.000	-.048	**.125**	.169	.116	.164	-.040	.181	.531	.278	.095	.112	.146	.283	.266	.071	.333	.301	-.048	.013	.108	-.020	.091
i. Number of weeks avocado was included in the infants diet	.176	-.108	.183	-.035	.113	-.093	.242	-.048	1.000	.236	**.345**	.296	.368	-.082	.299	.083	.244	.103	.274	.351	.432	.351	-.089	-.065	.364	.298	.259	.222	.391	.070
j. Number of weeks broccoli was included in the infants diet	.520	.214	.533	.112	.231	-.022	.134	.125	.236	1.000	.688	**.711**	.262	.080	.707	.207	.249	.200	.304	.540	.456	.475	.169	.259	.590	.504	.451	.383	.265	.238
k. Number of weeks carrots were included in the infants diet	.653	.264	.633	.191	.296	.111	.294	.169	.345	.688	1.000	.917	**.362**	.097	.903	.437	.446	.358	.336	.596	.468	.627	.162	.243	.770	.676	.702	.517	.321	.383
l. Number of weeks apples were included in the infants diet	.613	.240	.673	.147	.327	.078	.131	.116	.296	.711	.917	1.000	.381	**.072**	.870	.383	.401	.303	.400	.631	.457	.580	.164	.184	.760	.619	.699	.525	.296	.406
m. Number of weeks grapes were included in the infants diet	.142	.081	.210	.112	.123	-.040	-.023	.164	.368	.262	.362	.381	1.000	-.032	**.314**	.161	.202	292	.512	.263	.546	.270	-.040	-.045	.424	.148	.227	.305	.148	.302
n. Number of weeks bacon was included in the infants diet	.285	.447	.185	.484	-.025	-.054	.349	-.040	-.082	.080	.097	.072	-.032	1.000	.120	**.190**	.159	.040	.079	-.045	.117	.001	-.046	.286	.052	.043	.065	.048	-.097	.060
o. Number of weeks poultry was included in the infants diet	.698	.312	.320	.167	.238	.168	.258	.181	.299	.707	.903	.870	.314	.120	1.000	.378	**.360**	.254	.338	.574	.443	.616	.120	.324	.717	606	.602	.449	.352	.352
p. Number of weeks sausages were included in the infants diet	.281	.056	.183	.229	.511	.304	.546	.531	.083	.207	.437	.383	.161	.190	.378	1.000	.416	**.012**	.119	.357	.366	.471	-.031	.419	.564	.228	.326	.203	.074	.244
q. Number of weeks Marmite was included in the infants diet	.307	.013	.284	.237	.073	.097	.426	.278	.244	.249	.446	.401	.202	.159	.360	.416	1.000	.406	**.249**	.463	.476	.404	.040	.170	.606	.316	.287	.262	.130	.369
r. Number of weeks jam was included in the infants diet	.144	.042	.193	.046	-.055	.128	.171	.095	.103	.200	.358	.303	.292	.040	.254	.012	.406	1.000	.250	**.092**	.253	.306	.019	-.017	.392	.234	.157	.151	.011	.288
s. Number of weeks dried fruit was included in the infants diet	.012	-.132	.097	-.089	.118	-.033	.067	.112	.274	.304	.336	.400	.512	-.079	.338	.119	.249	.250	1.000	.461	.519	.304	.185	-.070	.508	.209	.114	.198	.189	.320
t. Number of weeks toddler packet snacks were included in the infants diet	.378	.043	.399	.098	.204	.047	.164	.146	.351	.540	.596	.631	.263	-.045	.574	.357	.463	.092	.461	1.000	.530	.473	.189	.122	.690	.419	.407	.271	.219	.327
u. Number of weeks raw fruit was included in the infants diet	.272	.111	.348	.289	.074	.095	.277	.283	.432	.456	.468	.457	.546	.117	.443	.366	.476	.253	.519	.530	1.000	.550	.019	.033	.584	.394	.275	.376	.260	.365
v. Number of weeks oily fish was included in the infant's diet.	.350	.022	.294	.051	.314	.257	.277	.266	.351	.475	.627	.580	.270	.001	.616	.471	.404	.306	.304	.473	.550	1.000	.056	.203	.723	.447	.409	.239	.293	.451
w. Number of weeks 'sweeties' were included in the infants diet	.119	-.040	.229	.006	-.018	.121	.016	.071	-.089	.169	.162	.164	-.040	-.046	.120	-.031	.040	.019	.185	.189	.019	.056	1.000	-.024	.207	.193	.041	.296	.229	-.003
x. Number of weeks crisps were included in the infants diet	.302	.253	.229	.088	.302	.273	.289	.333	-.065	.259	.243	.184	-.045	.286	324	.419	.170	-.017	-.070	.122	.033	.23	-.024	1.000	.211	.137	.025	.120	.073	.064
y. Number of weeks bread was included in the infants diet	.443	-.001	.460	.142	.303	.174	.367	.301	.364	.590	.770	.760	.424	.052	.717	.564	.606	.392	.508	.690	.584	.723	.207	.211	1.000	.458	.531	.416	.321	.435
z. Number of weeks butternut squash was included in the infants diet	.490	.199	.592	.127	.199	-.092	.041	-.048	.298	.504	.676	.619	.148	.043	.606	.228	.316	.234	.209	.419	.394	.447	.193	.137	.458	1.000	.479	.485	.251	.226
aa. Number of weeks parsnips were included in the infants diet	.502	-.011	.477	.129	.235	.126	.086	.013	.259	.451	.702	.699	.227	.065	.602	.326	.287	.157	.114	.407	.275	.409	.041	.025	.531	.479	1.000	.399	.150	.246
bb. Number of weeks sweetcorn was included in the infants diet	.417	.147	.452	.279	.210	.040	.091	.108	.222	.383	.517	.525	.305	.048	.449	.203	.262	.151	.198	.271	.376	.239	.296	.120	.416	.485	.399	1.000	.360	.214
cc. Number of weeks kiwi was included in the infants diet	.232	-.147	.116	-.067	.037	-.029	.142	-.020	.391	.265	.321	.296	.148	-.097	.352	.074	.130	.011	.189	.219	.260	.293	.229	.073	.321	.251	.150	.360	1.000	-.010
dd. Number of weeks oranges/citrus were included in the infants diet	.124	.024	.222	-.006	.226	213	.014	.091	.070	.238	.383	.406	.302	.060	.352	.244	.369	.288	.320	.327	.365	.451	-.003	.064	.435	.226	.246	.214	-.010	1.000

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Glossary

Allergen – any substance that can cause an allergy

Allergic disease – clinical conditions caused by allergy

Allergic march – the progression of one clinical manifestation of allergy to another. Normally starting with food allergy, progressing through eczema and asthma to allergic rhinitis

Allergic rhinitis – inflammation of the mucous membrane lining the nose caused by an allergy

Antigen – any substance that can cause an immune response in the human body

Asthma – respiratory disorder characterized by wheezing, usually of allergic origin

Atopy – a hereditary tendency to be hypersensitive to certain allergens

Atopic dermatitis – a severe condition of inflamed skin (eczema) characterised by atopy

Case control study – study with subjects sampled from the population of people with the outcome of interest

Cohort study – study with subjects taken from the population with different levels of exposure to factors of interest

Cytokine – a protein secreted by cells of the immune system that serve to regulate the immune system

Eczema – generic term for inflammatory conditions of the skin

Epigenetic – Something that affects a cell, organ or individual without directly affecting its DNA. An epigenetic change may indirectly influence the expression of the genome

Food Allergy – a hypersensitivity reaction to a food or food ingredient where immune mechanisms are involved.

Hydrolysed infant formula – infant formula that has been treated so the protein has been split into shorter length peptides

Immunoglobulin E (IgE) – the immunoglobulin involved mechanistically in IgE mediated food allergies

IgE mediated food allergy – a hypersensitivity reaction to a food or food ingredient where IgE can be demonstrated to have a role (either by skin prick test or serum specific IgE measurement).

Incidence – the observation of an event, usually the first clinical sign of disease

Non-IgE food allergy – a hypersensitivity reaction to a food or food ingredient where immune mechanisms are involved but IgE cannot be shown to be acting.

Prebiotic – non-digestible food ingredient that beneficially affect the host by beneficially stimulating growth and or activity of certain bacterial species in the colon

Prevalence – the presence or absence of disease at a point in time

Probiotics – live micro-organisms with various, strain specific immunomodulatory effects

Reverse causality – where an observed effect is due to the outcome measure, not the cause of it

Skin prick test – A non-invasive diagnostic procedure used to determine the presence of IgE. Provides a result within 15 minutes but is not 100% positively or negatively predictable

Serum Specific IgE – A blood measurement of levels of IgE which is specific to a certain antigen (as opposed to total serum IgE). Levels above 0.35kU_A/l, are taken as confirming allergy to that antigen

Validity – an expression of the degree to which a measurement is a true and accurate measure of what it purports to measure

Wheeze – breathing with a husky or whistling sound

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