THE EFFECT OF A COWS' MILK EXCLUSION DIET AND SUBSTITUTE FORMULA IN INFANCY ON CHILDHOOD EATING HABITS

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Abstract

Cows' milk allergy is the most common infant food allergy, requiring a strict exclusion diet usually for the first year of life. This exclusion of a main food group occurs at a critical time in the development of food preferences and eating habits. It is known that infants are born with an innate predisposition to prefer and reject certain tastes. However, these innate preferences can be altered with exposure to (and exclusion of) different foods. The aim of this research was to determine if the use of substitute formula and exclusion of milk products in the management of cows' milk allergy affects fussy eating and food preferences in the short or long term.

This research consisted of two separate cross-sectional studies that measured eating behaviours in children currently and previously consuming an exclusion diet for cows' milk allergy. Two different age groups were assessed; infants and school-aged children. The outcome measures were: fussy eating, food neophobia, feeding difficulties, dietary variety, nutritional intake, food preference, taste preference and growth.

The main findings were that children currently consuming an exclusion diet for cows' milk allergy have higher levels of fussy eating, feeding difficulties and food neophobia compared to a control group. These levels were associated with the number and type of allergic symptoms. Children who had consumed an exclusion diet for cows' milk allergy in infancy had significantly higher levels of avoidant eating behaviour and a lower preference for dairy products than the control group, several years after cows' milk had been reintroduced. Significant differences in dietary variety and the intake of some micronutrients were observed, but there was no difference in growth measurements between groups at either age.

This research has identified some novel findings, which have implications for health professionals and researchers working in food allergy and childhood nutrition. Whilst consuming an exclusion diet is essential for symptomatic relief in the management of cows' milk allergy, it is clear that it can have secondary effects on fussy eating and food preferences. These secondary effects can persist into adolescence. It therefore underlines the importance of ensuring exclusion diets for food allergy are not initiated unnecessarily or implemented for too long. The diagnosis of cows' milk allergy needs to be robust and monitoring of both exclusion diets and fussy eating should be timely.

Contents

Declaration

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

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Abbreviation list

AAF: Amino Acid Formula

ALSPAC: Avon Longitudinal Study of Parents and Children

BLW: Baby Led Weaning

BMI: Body Mass Index

BSACI: British Society of Allergy and Clinical Immunology

CEBQ Child Eating Behaviour Questionnaire

CFNS: Child Food Neophobia Score

CMA: Cows' Milk Allergy

CME: Cows' Milk Exclusion

DRV: Dietary Reference Value

DVS: Diet Variety Score

EAR: Estimated Average Requirement

EHF: Extensively Hydrolysed Formula

FA: Food Allergy

FAIR study: Food Allergy and Intolerance Research study

FFQ: Food Frequency Questionnaire

FHS: Food Hypersensitivity

GP: General Practitioner

IgE: Immunoglobulin E

IOW: Isle of Wight

NHS: National Health Service

NICE: National Institute of Health and Clinical Excellence

Non-IgE: Non Immunoglobulin E

SPT: Skin Prick Test

SIgE: Specific IgE

PIFA study: Prevalence of Infant Food Allergy study

PCA: Principal Component Analysis

RNI: Reference Nutrient Intake

NDNS: National Diet and Nutrition Survey

UK: United Kingdom

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Dissemination

Journal publications

- **Maslin, K.**, Dean, T., Arshad, S.H. & Venter, C. Cows' milk exclusion diet during infancy: Is there a long term effect on children's eating behaviour and food preferences? *Paediatric Allergy and Immunology*. Accepted November 2015.(appendix 1)

- **Maslin, K.**, Dean, T., Arshad, S.H. & Venter, C. (2015) Fussy eating and feeding difficulties in infants and toddlers consuming a cows' milk exclusion diet. *Paediatric Allergy and Immunology*, 26: 503-508.(appendix 2)

- **Maslin, K.**, Brown, T. & Venter, C. (2015) Colic – a guideline emphasising simple measures of support. *Nursing in Practice Journal*, February 2015.(appendix 3)

- **Maslin, K.**, Meyer, R., Reeves, L., Mackenzie, H., Swain, A., Stuart-Smith, W., Loblay, R., Groetch, M. & Venter, C. (2014) Food allergy competencies of dietitians in the United Kingdom, Australia and United States of America. *Clinical and Translational Allergy*, 4**:**37.(appendix 4)

- **Maslin, K.** & Venter, C. (2013) Developing a clear understanding of cow's milk protein allergy, *Journal of Family Health Care*; Spring 2013 Supplement, p8. (appendix 5)

- Venter, C., **Maslin, K.,** Patil, V., Grundy, J., Glasbey, G., Abid, R., Vlieg-Boerstra, B. & Dean, T. (2015) Validation and Acceptability of Food Challenge Recipes for use in Double-Blind, Placebo-Controlled Food Challenges in Children. *Paediatric Allergy & Immunology,* doi: 10.1111/pai.12454. [Epub ahead of print] (appendix 6)

- Venter, C. & **Maslin, K**. The Future of Infant and Young Childrens' Food: food supply, manufacturing and human health challenges in the 21st century. (2016) Nestlé Nutr Inst Workshop Ser, vol 85, pp 19–27, (DOI: 10.1159/000439479). (appendix 7)

Journal publications in preparation

- **Maslin, K.,** Dunn Galvin, A., Shepherd, S., Dewey, A., Dean, T. & Venter C. A qualitative study of mothers' perceptions of weaning and the use of commercial infant food in the United Kingdom.
- Venter, C., **Maslin, K.**, Patil, V., Kurukulaaratchy, R., Grundy, J., Glasbey, G., Dean, T. & Arshad S.H. The prevalence, natural history and time trends of peanut allergy over the first 10 years of life in two cohorts born in the same geographical location 12 years apart.
- Venter, C., **Maslin, K.**, Grundy, J., Glasbey, G., Pereira, B., Dean, T. & Arshad, S.H. Does breast-feeding whilst introducing solid foods prevent the development of food allergy?

Book chapters

- **Maslin, K.** & Venter, C. Food allergy in ABC Nutrition (5th edition). Wiley. In press.

Oral presentations

- **Maslin, K.** British Dietetic Association Food Allergy Interest Group Annual meeting. Cows' milk allergy: Beyond the exclusion diet. 3rd July 2015.
- **Maslin, K.**, Dean, T., Grundy, J., Glasbey, G., Arshad, S.H. & Venter, C. Consuming a cows' milk exclusion diet during infancy affects eating behavior and liking for dairy products 10 years later. American Academy of Allergy Asthma and Immunology Conference February 2015. *Journal of Allergy and Clinical Immunology*, Volume 135, Issue 2, Supplement, February 2015, Page AB72. Awarded travel scholarship for allied health professional category.

Poster presentations

- **Maslin, K.**, Dean, T., Arshad, S.H. & Venter, C. Do infants consuming a cows' milk exclusion diet have a healthier dietary pattern than infants consuming an unrestricted diet? Poster presentation at the British Society of Allergy and Clinical Immunology annual meeting, Telford, September 2015.

- Venter, C., **Maslin, K.** & Dean, T. A qualitative study of mothers' perception of weaning and getting the "allergy" message across. Poster presentation at the European Academy of Allergy and Clinical Immunology Conference, Barcelona, June 2015.

- **Maslin, K.**, Dean, T., Arshad, S.H. & Venter, C. Feeding difficulties, food neophobia and dietary variety in infants with Cows' Milk Allergy Poster presentation at the British Feeding and Drinking Group Conference, Portsmouth April 2014. *Appetite*, Volume 83, December 2014, Page 362.

- **Maslin, K.**, Dean, T., Arshad, S.H. & Venter, C. Dietary and Weaning Habits in Cows' Milk Allergy: A case series of 20 infants. Poster presentation at the European Academy of Allergy and Clinical Immunology Conference, Copenhagen, June 2014.

- **Maslin, K.,** Venter, C., Brown, T., Shah, N., Walsh, J. & Fox, A.T. Diagnosis and management of non-IgE-mediated cow's milk allergy in Infancy - a UK Primary Care practical guide. Poster presentation at the British Society of Allergy and Clinical Immunology annual meeting, Telford, July 2013.

Reports

- Dean, T., MacKenzie, H., Kilburn, S., Moonesinghe, H., Lee, K., **Maslin, K.** & Venter, C. (2013) Literature searches and reviews related to the prevalence of food allergy in Europe European Food Safety Authority.

Chapter 1: General introduction

1.1 Background

It is well known that diet and food exposure in early life can affect eating habits that track into later childhood and adolescence. Longitudinal research using birth cohort studies from the United Kingdom (UK) indicates that children's dietary habits may change gradually over time, but they are established early in life and are broadly stable throughout childhood (Frémeaux et al., 2011; Northstone, Smith, Newby, & Emmett, 2012). Early infancy is characterised as a sensitive period of time when learning about food preferences takes place. Infants need to be exposed to a variety of tastes and foods during this sensitive period in order to take advantage of the developmental plasticity and optimise the feeding experience (Beauchamp & Mennella, 2009; Cooke & Fildes, 2011; Forestell & Mennella, 2007; Schwartz, Scholtens, Lalanne, Weenen, & Nicklaus, 2011). Milk, whether breast milk or formula milk, is the first food that infants consume and becomes the standard against which all other new foods and flavours are evaluated (Birch & Doub, 2014). Therefore the type, mode and taste experience of milk feeding may modify food preferences, nutritional intake and subsequently growth.

Cows' milk allergy (CMA) is the most common infant food allergy in the UK. Infants with CMA who are not exclusively breastfed are prescribed substitute infant formula, composed either of hydrolysed peptides, amino acids or occasionally soya protein. These formulas have an altered taste that is commonly perceived as unpalatable. Additionally infants with CMA need to adhere to a strict weaning diet avoiding all forms of cows' milk in food, usually until at least one year of age. This management provides symptomatic relief to infants with CMA, however it is not known what the effect of consuming a substitute formula and cows' milk exclusion (CME) diet is on food preferences, eating habits, nutritional intake and growth in the short or long term.

Fussy eating is another common dietary problem of early childhood (Dovey, Staples, Gibson, & Halford, 2008;Taylor, Wernimont, Northstone, & Emmett, 2015). It may occur separately to or co-exist with CMA. Both problems are known to cause parental anxiety and may prompt parents to seek health professional advice. In food allergic children, problematic eating behaviours such as fussy eating and feeding difficulties can pose a particular nutritional dilemma, by further limiting the variety of an already restricted diet. It is not known whether fussy eating and feeding difficulties are more common in children with food allergy. This thesis will explore the link between these two matters and aim to bring some clarity to the association.

It will also evaluate any potential long-term consequences, thus making an original contribution to knowledge.

1.2 Aim and research questions

The overall aim of this PhD is to determine if consuming an exclusion diet for CMA in infancy has a short or long term effect on eating habits. In order to address this aim, two separate studies were undertaken in children of different ages.

The following research questions will be addressed in this thesis:

-Do children consuming an exclusion diet for CMA have different levels of fussy eating, feeding difficulties, food neophobia and dietary variety than children consuming an unrestricted diet? Does this affect their growth?

-Do children who consumed an exclusion diet for CMA in infancy have altered taste and food preferences in the long term, once cows' milk has been reintroduced into the diet? Does the early exposure to this diet affect their nutritional intake and growth in the long term?

1.3 Possible clinical implications

The results of this PhD will potentially have implications for children with diagnosed CMA, children with suspected but unproven CMA and non-affected healthy children. In addition to children with diagnosed CMA, it is known that some parents may incorrectly perceive their child to have a food allergy (Venter et al., 2006) and that CME diets are sometimes initiated unnecessarily (Eggesbo, Botten & Stigum, 2001; Sinagra et al., 2007). This implies that more children than necessary are likely to have a major food group excluded from their diet at a time in life that is critical for growth, development and establishment of eating habits. If consuming a CME diet is found to negatively affect eating behaviour, it provides a stronger argument for ensuring that children are diagnosed with CMA in a timely and accurate manner and followed up sufficiently for early detection of development of tolerance. The results of this PhD are expected to inform clinical dietetic practice regarding the optimum management of infant nutrition in CMA in the short term and highlight any potential outcomes of CME on long term eating habits. As this study will recruit two control groups of children who are consuming unrestricted diets, it will also explore the determinants and factors associated with fussy eating and feeding problems in general.

1.4 Thesis layout

Following on from this introductory chapter, there are two separate chapters reviewing the

literature related to i) cows' milk allergy and ii) the development of infant and child feeding behaviour. The first literature review chapter, chapter two, provides a detailed background to CMA, by outlining and reviewing its prevalence, presentation, natural history, dietetic management and nutritional implications. The second literature review chapter, chapter three, provides an overview of the many factors that may determine food acceptance and dietary patterns in infancy and childhood. The literature review search strategy is detailed in appendix 8.

Chapter four presents a study comparing the eating habits of infants and toddlers consuming a CME diet to a control group of children consuming an unrestricted diet. It specifically focuses on differences between the two groups in levels of fussy eating, feeding difficulties, food neophobia and dietary variety.

Chapter five describes a similar study to chapter four, but the participants are school aged children. This second study compares the eating habits of a group of children who consumed a CME diet during infancy to a control group who consumed an unrestricted diet during infancy. It specifically focuses on differences between the two groups in fussy eating, food preferences and taste preferences. The participants from this study were predominantly recruited from two prospective birth cohort studies. This study design therefore allows the long-term effects of a CME diet to be explored using prospectively collected infant feeding data.

Finally, chapter six explains the overall findings of this research by collating and discussing the results of study one and two together. The findings are discussed in the context of previous literature and implications for the management of CMA. Strengths and limitations of the research are addressed and future research needs are outlined.

Chapter 2: Literature review of cows' milk allergy

2.1 Overview of chapter

This chapter explores the prevalence, symptoms, diagnosis and management of CMA in infants and children, including the role of substitute formulas. It will also discuss the nutritional and growth implications of cows' milk exclusion (CME) diets, the role of dietetic input and the natural history of CMA, including incomplete resolution of symptoms.

2.2 Introduction to food hypersensitivity and food allergy

Food hypersensitivity (FHS) is an umbrella term that describes any reproducible adverse reaction to a food. It is subdivided into food allergy and non-allergic food hypersensitivity (commonly known as food intolerance). The main differentiating factor between food allergy and intolerance is that food allergy involves an immunological mechanism. Food allergy can be further subdivided into two categories based on the pathophysiological processes involved: those involving immunoglobulins (IgE) and those not involving immunoglobulins (non-IgE mediated). Non-allergic food hypersensitivity can be subdivided into three further categories. This is shown in figure 2.1, adapted from Johansson et al. (2004)

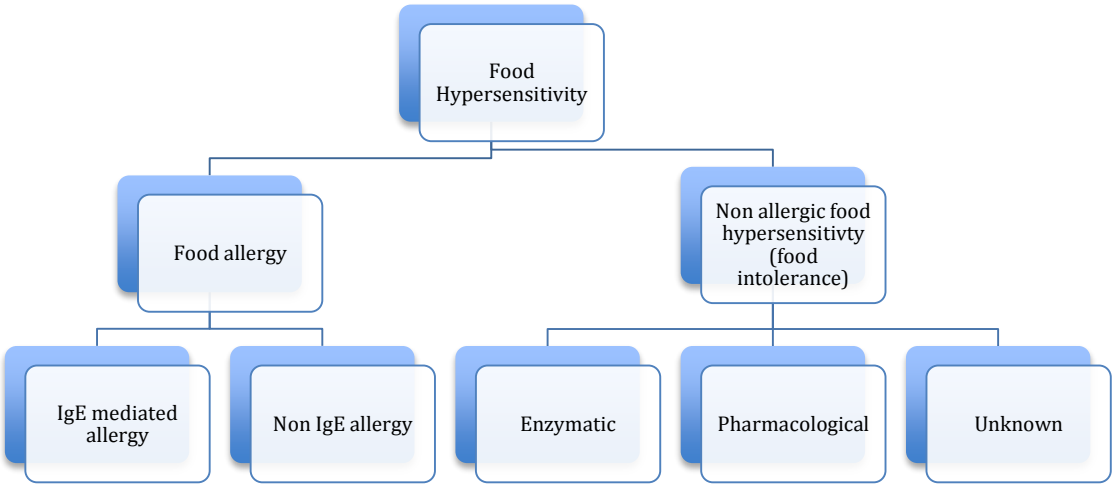

Figure 2.1 Nomenclature of food hypersensitivity

Food allergy can be caused by a wide range of foods, but eight foods (cows' milk, hens' egg, soy, peanuts, tree nuts, wheat, fish, and shellfish) account for more than 90% of childhood

cases (Boyce et al., 2010). Studies estimating the prevalence of food allergy vary significantly depending on the country, method of diagnosis (e.g. self-reported, doctor-diagnosed or challenge-proven), study design and the study population. Symptoms in both adults and children are often wrongly attributed to food allergy; therefore a robust diagnostic process is needed for confirmation. At present there is no cure for food allergy; management requires the individual to consume an exclusion diet avoiding the causative allergen. Therefore the main goals in the management of food allergy in children are to prevent the occurrence of acute and chronic symptoms by avoiding the offending food(s), whilst providing an adequate, healthy and nutritionally balanced diet and maintaining optimal growth; ideally under the guidance of a trained dietitian (Venter, Laitinen, & Vlieg-Boerstra, 2012).

2.3 Definition and epidemiology of cows' milk allergy

CMA is a reproducible adverse reaction to one or more milk proteins, involving the immune system. The main allergens in cows' milk are alpha-lactalbumin, beta lactoglobulin, bovine serum albumin, alpha casein and kappa casein (Fiocchi et al., 2010). Data derived from birth cohort studies using objective diagnostic methods estimate that approximately 1.9%-4.9% of children have CMA (Fiocchi et al., 2010), with the prevalence in adults much lower; less than 0.5% (Venter & Arshad 2011). A meta-analysis of studies published from 1990-2005 worldwide found a prevalence 0-3% of challenge-proven CMA, however there were insufficient studies available for inclusion in older children (Rona et al., 2007).

In Europe, a more recently published meta analysis of studies from 2000 to 2012 determined the overall lifetime prevalence of self-reported CMA to be 6%, but only 0.6% prevalence using an objectively verified method of food challenge diagnosis (Nwaru et al., 2014). One in twenty parents reported their child to have a food allergy, with dairy products being the most commonly reported allergen. Studies included in this meta-analysis were found to have a moderate level of bias, with differences in participation rates and methodology. Looking specifically at data from the UK, birth cohort studies using objective diagnoses estimate CMA to affect 1.26- 2.9% of young children (Schoemaker et al., 2015; Venter et al., 2008). Unlike some other European countries, it is estimated that the majority of CMA in the UK is non-IgE mediated (Schoemaker et al., 2015; Venter et al., 2006).

2.4 Symptoms of cows' milk allergy

CMA typically presents in the first few months of life when the infant is exposed to cows' milk; either transmitted via breast milk, infant formula or in solid food. Symptoms can range from mild

to severe and can manifest as a number of different clinical characteristics, mainly affecting the skin, gastro-intestinal tract and respiratory systems. This is summarised in Table 2.1. It is estimated that most infants with CMA have more than two symptoms and symptoms from more than two organ systems (Host, Jacobsen, Halken, & Holmenlund, 1995; Schoemaker et al., 2015).

Patterns of reactivity can vary due to different levels of exposure and time intervals between exposures. The onset of symptoms may be immediate, delayed or a combination, depending on whether the allergy is IgE mediated, non-IgE mediated or both. Symptoms of IgE CMA occur instantly or within an hour, whereas symptoms of non-IgE mediated CMA usually occur between 2-48 hours after exposure. Individuals with mixed IgE and non-IgE CMA can have both chronic and acute symptoms. In severe cases CMA can cause systemic reactions, accounting for 11–28% of anaphylaxis reactions, including up to 11% of fatal reactions (Fiocchi et al., 2010).

Table 2.1 Signs and symptoms of cows' milk allergy

IgE-mediated (Immediate)	**Non-IgE-mediated (delayed)**
The skin	
• Pruritus • Erythema • Acute urticaria • Acute angioedema (most commonly in the lips and face, and around the eyes)	• Pruritus • Erythema • Atopic eczema
The gastrointestinal system	
• Angioedema of the lips, tongue and palate • Oral pruritus • Nausea • Colicky abdominal pain • Vomiting • Diarrhoea	• Gastro-oesophageal reflux disease • Loose or frequent stools • Blood and/or mucus in stools • Abdominal pain/ Infantile colic • Food refusal or aversion • Constipation • Perianal redness • Pallor and tiredness • Faltering growth plus one or more gastrointestinal symptoms above (with or without significant atopic eczema)
The respiratory system (usually in combination with >1 of the above symptoms/signs)	
• Upper respiratory tract symptoms – nasal itching, sneezing, rhinorrhoea or congestion (with or without conjunctivitis)	
• Lower respiratory tract symptoms (cough, wheezing or shortness of breath)	
Other	
• Signs or symptoms of anaphylaxis or other systemic allergic reactions	

Adapted from NICE guidelines (NICE, 2011)

An expert panel of clinicians recently reviewed the literature regarding CMA to determine

whether a clinical score derived from symptoms associated with the ingestion of cows' milk proteins could help with the diagnosis of CMA (Vandenplas et al., 2015). The Cow's Milk-related Symptom Score (CoMiSS), was developed as an awareness tool for cows' milk-related symptoms. It includes a combination of dermatological, gastrointestinal and respiratory symptoms. Each symptom has a maximum score of six, apart from respiratory symptoms, which have a maximum score of three. An arbitrary cut-off value of ≥12 was selected as the criterion to detect infants at risk of CMA. Although this scoring system could be used to assess the improvement of symptoms following dietary elimination, it is not a diagnostic tool and has not yet been validated.

2.5 Diagnosis of cows' milk allergy

Symptoms suggestive of CMA can be non-specific and characteristic of other childhood diseases. These symptoms can occur in 5-15% of children (Høst & Halken, 2014), emphasising the importance of ensuring an early and correct diagnosis to prevent on-going symptoms and unnecessary dietary restrictions. It is not uncommon for parents to suspect that their infant is allergic to a food, which in the majority of cases will not be confirmed by a medical diagnosis (Venter et al., 2006).

There is no single diagnostic test for CMA, nor is there evidence to support the use of IgG testing, hair analysis, vega testing or kinesiology (Boyce et al., 2010; Luyt et al., 2014; NICE, 2011). The gold standard for diagnosis of any food allergy is a double blind placebo controlled food challenge (Sampson et al., 2012). However in routine clinical practice, this is rarely undertaken and is usually reserved for research. In clinical practice, diagnosis usually takes place using a detailed clinical history, followed by allergy tests if appropriate and/or an exclusion diet, followed by hospital or home reintroduction (Luyt et al., 2014;Venter, Brown, Shah, Walsh, & Fox, 2013).

2.6 History taking in cows' milk allergy

Adverse reactions to cows' milk can occur due to IgE- and/or non-IgE-mediated reactions or non-immunologic reactions (e.g. primary or secondary lactose intolerance). A thorough clinical history allows the aetiology of reactions to be differentiated. The UK National Institute of Clinical Excellence (NICE) (2011) guidelines emphasise that CMA should be particularly considered in: infants with a family history of allergic disease, where symptoms are persistent and affecting different organ systems and in infants who have been treated for moderate to severe atopic eczema, gastro-oesophageal reflux disease or other persisting gastrointestinal symptoms

(including ‘colic’, loose stools, constipation), but have not responded to the usual initial therapeutic interventions. Taking an allergy-focused history forms the cornerstone of the diagnosis of CMA and it is recommended that questions should be asked regarding (Fiocchi et al., 2010; Skypala et al., 2015; Venter et al., 2013).

• Any family history of atopic disease in parents or siblings.

• Any personal history of early atopic disease.

• The infant's feeding history; whether breast-fed or formula fed and timing of weaning (if commenced).

• Presenting symptoms and signs that may be indicating possible CMA.

• Details of previous management or dietary avoidance and any response to these interventions.

If the allergy-focused history strongly suggests that cows’ milk may be a causative factor, allergy tests and/or an exclusion diet with reintroduction are the next step. The history of symptoms will inform whether IgE or non-IgE mediated CMA is most likely.

2.7 Diagnosis of IgE- mediated cows’ milk allergy

A diagnosis of IgE-mediated CMA requires both sensitisation, meaning the presence of allergen-specific antibodies, and clinical symptoms after exposure to cows’ milk. The use of a skin prick test (SPT) or specific IgE (blood) tests to detect the presence of antibodies are recommended, but these should only be performed by those with the competencies to interpret the test (NICE, 2011). They should not be used as a screening tool due to their poor predictive ability. A positive SPT or specific IgE test merely indicates the presence of antibodies and does not confirm clinical allergy. However, a positive test coupled with a very clear history of a reaction may be able to confirm a diagnosis, although an oral food challenge (after a period of cows’ milk avoidance) in a hospital setting will be required in many cases to confirm the diagnosis.

2.8 Diagnosis of non-IgE mediated cows’ milk allergy

There are no validated tests for the diagnosis of non-IgE mediated CMA, apart from strict avoidance of cows’ milk for approximately 4-6 weeks, followed by reintroduction to confirm the diagnosis (Luyt et al., 2014; Venter et al., 2013). Home challenges may not be acceptable in children with severe forms of non-IgE mediated cow’s milk allergy (e.g. if food protein enterocolitis is suspected). In exclusively breastfed infants, elimination and challenge should take place via restriction of maternal intake of cows’ milk under the supervision of a dietitian.

An algorithm outlining the primary care diagnosis and management of mild to moderate non-IgE CMA for use in the UK is shown in Figure 2.2 (Venter et al., 2013).

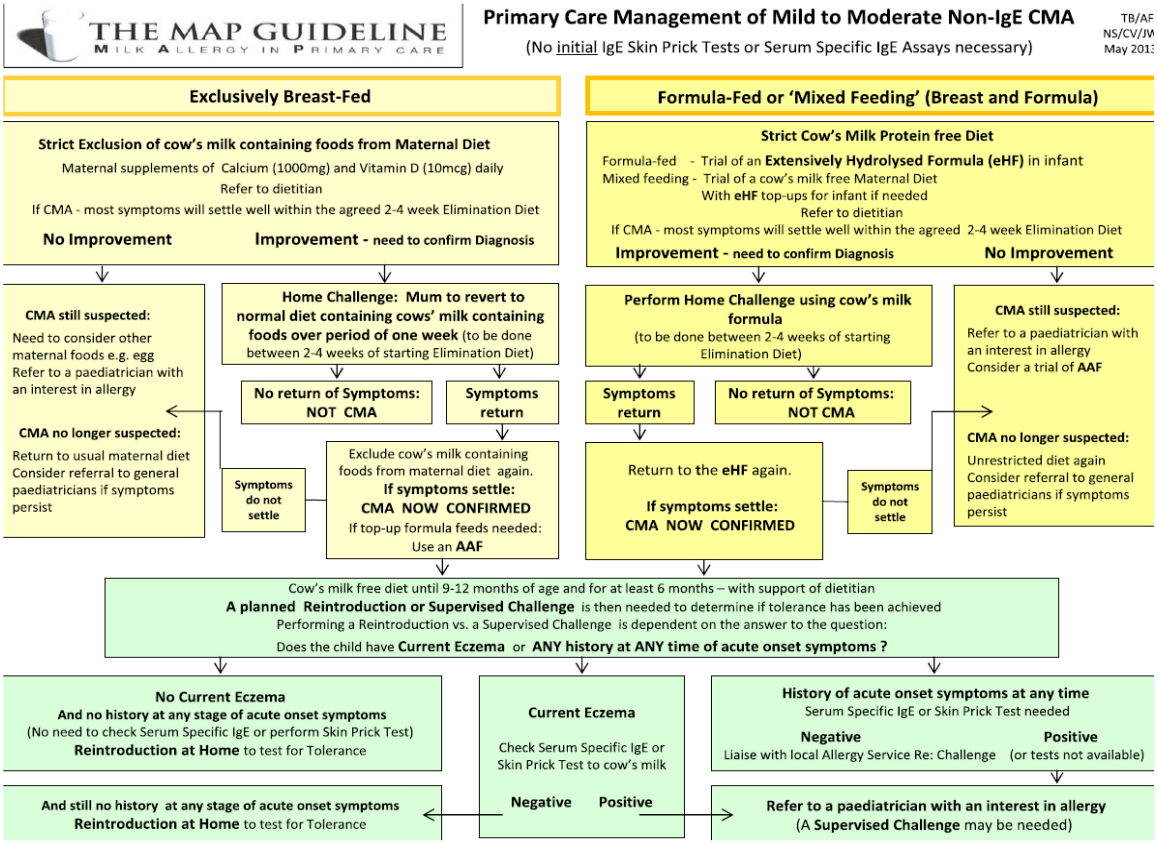

Figure 2.2 Algorithm for diagnosis and management of mild-moderate non-IgE CMA

2.9 Substitute formula used in cows' milk allergy

2.9.1 Extensively hydrolysed and amino acid formulas

A number of substitute infant formulas are available for the diagnosis and management of CMA, which can be used on their own or in combination with breastfeeding. These can be broadly separated into two main categories: extensively hydrolysed formula (EHF) and amino acid formula (AAF). EHF are either casein or whey derived and have been enzymatically treated to break the protein into smaller peptides. Casein EHF are more hydrolysed than whey EHF, with greater than 90% of the peptides being smaller than 1.5kDA. AAFs are composed of pure synthetic amino acids and are the only formula for CMA that can be considered to be non-allergenic.

Extensively hydrolysed proteins derived from cows' milk have been used in formulas for more than sixty years in infants with CMA (Høst & Halken, 2014). The allergenicity of cows'

milk is reduced by enzymatic hydrolysis or by a combination of hydrolysis, heat treatment, and/or ultra- filtration (Høst et al., 1999). This process reduces the number of conformational and sequential epitopes, thus reducing the allergenicity. There are two different definitions for hypoallergenic formula for use in CMA. Firstly, European Union regulations for labelling infant formulas as hypoallergenic are based arbitrarily on an immunoreactive protein content of < 1% of total nitrogen containing substances (Høst et al., 1999). A second definition is that hypoallergenic formula should be tolerated by at least 90% of infants with documented CMA (with 95% confidence) (Høst et al., 1999). The most commonly used EHF and AAF in the UK for the management of CMA are shown in Table 2.2, adapted from Meyer & Venter (2014).

Table 2.2 Commonly used EHF and AAF in the management of CMA in the UK.

Name	Type	Molecular weight of proteins
Aptamil Pepti 1 and 2	EHF whey	50.5% peptides < 500 Da 73% of peptides < 1000 Da
Althera	EHF whey	92% peptides < 500 Da 99.3% peptides < 1000 Da
Nutramigen LIPIL 1 and 2	EHF casein	60.4% peptides < 500 95% peptides < 1000 Da
Similac Alimentum	EHF casein	73% peptides < 500Da 95% peptides < 1000 Da
Infatrini Peptisorb	EHF whey	47.3% peptides < 500 Da 70.5 % peptides <1000 Da
Neocate LCP	AAF	100% Amino acids
Alfamino	AAF	100% Amino acids
Puramino Nutramigen	AAF	100% Amino Acids
Neocate Active*	AAF	100% Amino acids
Neocate Advance*	AAF	100% Amino acids

*for children > 1 year old.

2.9.1.1 Indications for extensively hydrolysed and amino acid formulas

Although the majority of infants with CMA will tolerate an EHF, some infants may react as EHFs possess residual allergenicity (Hill, Murch, Rafferty, Wallis, & Green, 2007). Choosing the most appropriate formula for the infant based on the clinical presentation is a matter of debate with differences in practice between centres and countries. The different indications for use of EHF and AAF according to several international guidelines are shown in Table 2.3 (Boyce et al., 2010; Fiocchi et al., 2010; Koletzko et al., 2012; Luyt et al., 2014). The choice should ultimately be based on clinical presentation, nutritional composition and residual allergenicity of the formula, although the palatability of the formula and age of the infant will be important factors. CMA is a heterogenous condition with a variation in type, number, severity and duration of symptoms and the presence of coexisting allergens; therefore the decision is not always straightforward. It will also depend on the availability/cost of formulas, local policies and the knowledge of the health professional involved.

Because EHFs by definition will improve symptoms in 90% of infants with CMA, they should be the first line treatment in most situations. In general EHF are indicated for mild to moderate presentations of CMA, with AAF reserved for more severe presentations of CMA (Venter et al., 2013), as indicated in Table 2.3 (from Meyer & Venter, 2014). A systematic review of twenty studies evaluating AAF and EHF identified specific subgroups of infants with CMA who would benefit from an AAF rather than an EHF. This included non-IgE mediated food induced proctitis syndromes with failure to thrive, multiple food allergy, severe atopic eczema or symptoms during exclusive breastfeeding; however the review also found inconsistent data reporting in studies therefore was unable to conduct a meta-analysis (Hill et al., 2007). In practice an increasing proportion of infants are being inappropriately prescribed an AAF. Taylor et al. (2010) reported no significant difference in distribution and severity of symptoms between infants prescribed an AAF and an EHF in a study of general practitioners (GPs) in the UK. However those who were treated with an AAF had significantly more GP visits than those treated with an EHF (17 vs 13 visits over one year), suggesting a difference in health care needs.

Table 2.3 Indications for substitute formulas for CMA according to selected national and international guidelines

Presentation	**World Allergy Organisation** (Fiocchi et al., 2010)	**British Society for Allergy and Clinical Immunology guidelines** (Luyt et al., 2014)	**European (ESPGHAN) guidelines** (Koletzko et al., 2012)	**USA (NIAID) guidelines** (Boyce et al., 2010)
Anaphylaxis	AAF	No recommendation	AAF	No recommendation
Acute urticaria or angioedema	EHF	No specific mention but EHF in general as first line treatment apart from specific indications for AAF	No specific mention but EHF in general as first line treatment apart from specific indications for AAF	No recommendation
Atopic eczema/dermatitis	EHF	No specific mention but EHF in general as first line treatment apart from specific indications for AAF	No specific mention but EHF in general as first line treatment apart from specific indications for AAF	No recommendation
Immediate gastrointestinal allergy	EHF	No specific mention but EHF in general as first line treatment apart from specific indications for AAF	No specific mention but EHF in general as first line treatment apart from specific indications for AAF	No recommendation
Allergic eosinophilic oesophagitis	AAF	AAF	AAF	AAF/hypoallergenic formula
Gastroesophageal reflux disease	EHF	No specific mention but EHF in general as first line treatment apart from specific indications for AAF	No specific mention but EHF in general as first line treatment apart from specific indications for AAF	No recommendation
Cows' milk protein-induced enteropathy	EHF	AAF	AAF	EHF/AAF
Food protein induced enterocolitis syndrome	EHF	AAF	AAF	Hypoallergenic formula
Cows' milk protein induced gastroenteritis and proctocolitis	EHF	No specific mention but EHF in general as first line treatment apart from specific indications for AAF	No specific mention but EHF in general as first line treatment apart from specific indications for AAF	No recommendation
Severe irritability (colic)	EHF	No specific mention but EHF in general as first line treatment apart from specific indications for AAF	No specific mention but EHF in general as first line treatment apart from specific indications for AAF	Hypoallergenic formula

2.9.1.2 Palatability of extensively hydrolysed and amino acid formula

Although substitute formulas are the only form of nutrition available for infants with CMA who are not breastfed, a key disadvantage is their poor palatability. The extensive proteolysis of milk leads to the formation of bitter and sour tasting peptides which have a volatile odour, meaning these formulas are perceived as unpalatable by infants, children and adults alike (Mennella, Griffin, & Beauchamp, 2004; Miraglia Del Giudice et al., 2015; Pedrosa Delgado, Pascual, Larco, & Martín Esteban, 2006; Sausenthaler et al., 2010). Attempts have been made to improve the palatability of some formula by inclusion of sucrose or lactose (Borschel & Baggs, 2015; Niggemann et al., 2008) and acceptance is often used as a key indictor of success in studies of their usage (Borschel & Baggs, 2015; Mabin, Sykes, & David, 1995; Rapp et al., 2013).

Studies of palatability have been conducted in different age groups. Infant studies, using analysis of facial expressions and mothers' judgments of infants' acceptance, have demonstrated that infants older than seven months dislike and reject these formula, but are more likely to be accepting if they have been exposed to them at a younger age (Mennella et al., 2004). A study in ten year old children (n =833) reported that most children evaluated hydrolysed formulas as "extremely bad" (Sausenthaler et al., 2010), although of course it must be noted that these formulas are not intended for use in children of this age. Two palatability studies have been undertaken recently in adults; one in Spain (n= 50) (Pedrosa Delgado et al., 2006) and one in Italy (n = 150) (Miraglia Del Giudice et al., 2015). Both studies evaluated a number of different categories of substitute formulas for taste, smell and texture; finding the casein hydrolysate performed the worst overall. Pedrosa et al. (2006) found a statistically significant correlation between peptide weight and taste rating, indicating the more hydrolysed a formula was, the more unpleasant it was rated. However it was also reported that formulas within the same category were rated differently, meaning other factors besides level of hydrolysis may influence the taste, such as the addition of sweeteners or flavourings.

Following on from this finding, Miraglia et al. (2015) determined that types of fat and carbohydrate were also related to palatability. They reported that palatability decreased with increasing levels of total polyunsatured fatty acids and maltodextrines, but increased with increasing levels of saturated fatty acids and lactose. Whilst both of these adult studies are interesting, it should be emphasised that the participants in these studies were not trained sensory panelists. In addition the relevance and clinical utility of testing products designed for infants in adults needs to be questioned. Taste preferences in infants and sensory testing in children will be discussed in more detail in chapters three and five.

2.9.2 Soya formula

Soya formula is not recommended for infants under six months of age, due to the quantity of isoflavones that will be consumed per kg of body weight in this age group (Committee on Toxicity of Chemicals in Food Consumer Products and the Environment, 2013; Committee on Toxicity of Chemicals in Food, 2003) and the risk of concomitant soya allergy in infants with CMA (Fiocchi et al., 2010; Luyt et al., 2014; Venter et al., 2013). Soya formula can however be used in infants not allergic to soya after the age of six months and may be useful in a situation where EHF is rejected due to poor palatability. Additionally soya-based weaning foods have a lower dose of isoflavones than soya-based formula, and can be a useful source of nutrients in infants with CMA (Committee on Toxicity of Chemicals in Food Consumer Products and the Environment, 2013). Prior to the guidance in 2003 advising against the use of soya formula in children under six months old, it was widely used in infants with CMA. This is relevant to this thesis as some of the participants were born prior to 2003, which will be discussed further in chapter five.

2.9.3 Alternative milks used in cows' milk allergy

Typically substitute formulas for CMA are indicated until the age of two years. As will be discussed in the upcoming section on the natural history of CMA, by this age most children will tolerate cows' milk. Due to inadequate levels of protein and calories, alternative plant based milks (e.g. oat, coconut, almond milk) are not recommended as a substitute drink for children under one year old and ideally not in children under two years old (Luyt et al., 2014).

Case reports have highlighted the danger of severe nutritional deficiencies that arise in infants consuming a plant based milk substitute. A French study (Le Louer et al., 2014) identified nine cases of infants aged from four to 14 months where plant based milks (rice, almond and chestnut milks) were used for presumed CMA for up to three months. Consequences reported included protein-calorie malnutrition with substantial hypoalbuminemia, diffuse oedema, severe hypocalcemia, severe iron deficiency anaemia, nutritional rickets and severe hyponatremia. It should be emphasised however that the exclusion diet in these cases was implemented without health professional advice or supervision. The nutritional consequences of consuming a CME diet and role of dietetic input will be discussed later in this chapter.

2.9.4 Breastfeeding

Breastfeeding is recommended where possible in infants with CMA (Fiocchi et al., 2010). In infants who are partially or exclusively breastfed and diagnosed with CMA, a maternal CME

diet is usually recommended. Suitable replacement products and adequate calcium and vitamin D supplements are recommended to ensure both mother and baby are nutritionally replete (Venter et al., 2013). A recent Cochrane review on maternal dietary exclusion during pregnancy and lactation concluded that adherence to an antigen avoidance diet during lactation requires considerable effort and there is a paucity of information regarding women's experience and compliance with such diets (Kramer & Kakuma, 2014).

2.10 Management of cows' milk allergy

The management of CMA in infants and young children requires individualised advice regarding avoidance of cows' milk, including advice to breastfeeding mothers and/or guidance on the most appropriate specialised formula or milk substitute (NICE, 2011). In many cases micronutrient supplements will also be required, however their usage is not always intuitive with both under and over supplementation occurring (Meyer et al., 2015). Often this needs advice and review from a paediatric dietitian trained in food allergy to ensure appropriate substitutes are chosen in order to optimise the nutritional content of the diet.

In most situations very strict avoidance is required (i.e. exclusion of trace amounts of cows' milk in processed products), requiring parents to carefully read food labels to identify possible sources of cows' milk, thus preventing accidental exposure. Foods and ingredients containing cows' milk are listed in Table 2.4. However, it is now known that the majority of children with CMA *may* tolerate heated milk and indeed inclusion of baked milk may accelerate the resolution of CMA in some children (Kim et al., 2011). The level of exclusion required can be determined by an allergy dietitian; taking into account the clinical history, age, quality of life and nutritional status of the patient.

Table 2.4 Ingredients and foods containing cows' milk.

Foods and ingredients that contain cows' milk protein
Butter, butter fat, butter milk, butter oil
Casein, caseinates, hydrolysed casein, calcium caseinate, sodium caseinate.
Cheese, cheese powder, cottage cheese.
Cows' milk (fresh, condensed, dried, evaporated, powdered (infant formulas), UHT)
Cream, artificial cream, sour cream
Ghee
Ice cream
Lactalbumin, lactoglobulin
Low-fat milk
Malted milk
Margarine
Milk protein, milk powder, skimmed milk powder, milk solids, non-fat dairy solids, non-fat milk solids, milk sugar
Whey, hydrolysed whey, whey powder, whey syrup sweetener
Yogurt, fromage frais

Adapted from BSACI guidelines (Luyt et al., 2014)

2.10.1 Role of dietetic input in the diagnosis and management of cows' milk allergy

To ensure effective management of any type of FHS disorder, an appropriate dietary assessment and avoidance strategy is required, particularly so with infants and children during a time of rapid growth and development (Groetch & Nowak-Wegrzyn, 2013). A knowledgeable and competent food allergy dietitian is uniquely qualified to deliver this. In recent years, four official international guidelines have been published on the diagnosis and management of food allergies recognising that dietitians play a role in both the diagnosis and management of food allergies; the UK National Institute of Health and Clinical Excellence guidelines on the diagnosis of food allergies in children (NICE, 2011), the European Society for Paediatric Gastroenterology, Hepatology and Nutrition guidelines on Cow's Milk Protein Allergy (Koletzko et al., 2012), the British Society of Allergy and Clinical Immunology (BSACI) guidelines for the diagnosis and management of cows' milk allergy (Luyt et al., 2014) and the Irish Food Allergy Network Paediatric Food allergy guidelines (Irish Food Allergy Network, 2012).

In practice, the role of a dietitian working in the area of food allergy involves a range of responsibilities, consisting of, but not limited to; taking an allergy-focused diet history and

interpretation of skin prick tests, advising on formula choice and complementary feeding including nutrient supplements, allergen avoidance advice including practical advice on substitutes and recipes, identifying any additional feeding problems either behavioural or nutritional, assessing nutritional intake and nutritional status, assistance with design of food challenges and advising pregnant and lactating women following an avoidance diet for more than a few weeks (Venter et al., 2012). Whilst excluding a food may be necessary to alleviate chronic allergic symptoms, it may increase the risk of an acute reaction upon reintroduction after long-term avoidance (Groetch & Nowak-Wegrzyn, 2013). It is therefore not without risk and requires expert management and advice.

Although recommended by a number of national and international guidelines, there is limited evidence of the effectiveness of dietetic intervention on the nutritional status of food allergy patients. Four quantitative and one qualitative studies were identified. Firstly Berni-Canani et al. (2014) conducted a multicenter intervention study in Italy in children aged one to three years. The study consisted of a food allergy group who were consuming an elimination diet without dietary counseling for at least two months (n = 91) and a non-matched control group of children without food allergy (n = 66). At baseline evaluation, children in the dietary elimination group had lower energy, protein, zinc and calcium intakes than the control group and were more likely to have poor growth. However six months after receiving nutritional advice, the total energy intake of children with food allergy was similar to the control group of children, resulting in a significant improvement of anthropometric and laboratory biomarkers of nutritional status. The improvement was not influenced by age, sex, allergen, single or multiple food allergy, duration of exclusion diet before enrolment, age at diagnosis, symptoms, or type of formula. The study was limited by the fact that the control group was not re-evaluated at six months, however overall it demonstrates the value of specialist nutritional advice.

Three further studies have highlighted the importance of dietary counselling. Christie et al. (2002) indicated that infants consuming exclusion diets who had not received nutritional advice were likely to have diets deficient in vitamin D and calcium, compared to those who had received nutritional advice. In the UK, a pilot study indicated that dietary advice given to food allergic infants from a specialist paediatric allergy dietitian as part of a multidisciplinary allergy clinic improved weaning practices, growth and dietary adequacy in food allergic infants (Tarkin & Meyer, 2013); however this study did not have a control group to compare against. Madsen & Henderson (1997) demonstrated that a single nutrition counselling session in children with inadequate calcium intake secondary to consuming a CME diet (n = 31) led to an increase of 360mg calcium/day when reassessed two years later, however 48% of children still did not

meet their requirements. This implies nutritional input has a beneficial effect, but one advisory session may be inadequate. Finally, qualitative research has indicated that parents value a range of support from dietitians, including monitoring their child's health and providing information, practical advice and support, in addition to emotional support. Specifically a focus group study of mothers recommended that dietetic advice should be provided as soon as possible after diagnosis and reviewed regularly and at important milestones (Mackenzie, Grundy, Glasbey, Dean, & Venter, 2015). Taken together all of these studies underline the importance of nutritional advice in optimising exclusion diets.

2.11 Nutritional consequences of exclusion diets

Cows' milk and its associated products, such as yoghurt and cheese, are sources of protein, energy, vitamin A, vitamin D, riboflavin, pantothenic acid, vitamin B12, calcium, phosphorous and iodine. Exclusion will therefore impact the diet if suitable substitutes and/or nutritional supplements are not used as replacements. Additionally, because cows' milk is a ubiquitous ingredient present in a range of processed foods (e.g. ham, biscuits, pasta sauce, soups), exclusion will have a further impact on food choice. Research to date has focused on the negative effect exclusion of cows' milk can have (Berry et al., 2015; Christie, Hine, Parker, & Burks, 2002; Flammarion et al., 2011; Henriksen, Eggesbø, Halvorsen, & Botten, 2000; Mabin et al., 1995; Tiainen, 1995). It is possible however that exclusion of cows' milk could inadvertently lead to healthier eating habits being adopted if suitable substitutes are chosen (e.g. eating fruit rather than a biscuit as a snack).

A systematic review of nutrient intake and growth in children with IgE-mediated food allergy identified six studies, but only three included nutritional assessment (Sova et al., 2013). A literature search identified four studies which have been conducted in a UK population (Devlin, Stanton & David, 1989; Mabin, Sykes & David, 1995; Noimark & Cox, 2008, Meyer et al., 2014). However, three of the studies did not include a control group and the fourth article describes a case series of three patients with misdiagnosed food allergy (Noimark & Cox, 2008). Studies conducted outside the UK have assessed the diets of infants and children with single and multiple food allergies, demonstrating differences in protein, calcium, zinc, vitamin D and iron intakes (Christie, Hine, Parker, & Burks, 2002; Flammarion et al., 2011; Henriksen, Eggesbø, Halvorsen, & Botten, 2000; Tiainen, 1995).

Tables 2.5 and 2.6 provide a summary of studies that have investigated nutritional intake in children consuming exclusion diets for food allergy. They have been divided into two tables, depending on whether they included a control group or not. The heterogeneity of the

studies makes comparison and definite conclusions difficult as studies have been undertaken in different age groups and settings, including both single and multiple exclusion diets with different study designs. Overall most studies report similar energy intakes between groups, indicating that excluded food groups are adequately substituted in terms of calories, although not always in terms of micronutrients.

Table 2.5 Studies (with a control group) assessing nutritional intake of children consuming exclusion diets

Authors, year, country	Age of participants	N	Method	Dietary exclusion	Main nutritional outcomes
Flammarion et al. (2011) France.	4.7 years	96 food allergic 95 paired controls	3 day food diary	41 excluded 3 foods 28 CMA	-Energy, protein and calcium intakes were similar between the two groups, and not any different dependent on number of foods excluded. -Vitamin D intake was similar between both groups, but below recommended levels. -In the 28 CMA children, 14 received a calcium supplement. Intake of calcium from food lower than control group, but met recommended levels with addition of supplement. -Energy intake similar between groups. Protein intake of exclusion group < than controls, but within normal range.
Berni-Canani et al. (2015) Italy	Mean 18.9 months (range 6-36 months)	91 food allergic 66 controls	3 day food diary	80 had CMA 42 multiple foods excluded	-Children in the dietary elimination group had lower energy, protein, zinc and calcium intakes than controls. -Of the 80 children with CMA, ten were not receiving any hypoallergenic substitute formula.
Christie et al. (2002) USA	3.7 years (range 1 month -10 years)	98 food allergic 99 controls	3 day food diary	45 multiple FA 20 single FA 20 CMA	-More children with FA did not meet recommended intake of calcium compared with control children. -91% of participants who had a substitute formula met recommended intakes for calcium and vitamin D.
Henriksen et al. (2000) Norway	2.5 years (range 31-37 months)	34 total	4 day weighed food record	16 milk free. 8 low milk consumption 10 in control group (egg free).	-Energy, protein, calcium, niacin and riboflavin intake lower in milk exclusion compared to milk consuming group. Mean intake of substitute formula lower than mean intake of cows' milk -4/10 of milk exclusion group received a calcium supplement, but two still had inadequate intake.
Tiainen et al. (1995) Finland	2 years (1-3.5 years)	18 CMA and 20 matched controls	6 day food diary	Cows' milk	-No difference in energy intake between groups -Lower protein and higher fat in CMA children. -Volume of formula consumed by CMA group was less than volume milk consumed by control group. -Mean energy, zinc and iron less than requirements in both groups. -11 children in CMA group and 14 in control group took vitamin A and D supplements.

Table 2.6 Studies (without a control group) assessing nutritional intake of children consuming exclusion diets

Authors, year, country	Age of participants	N	Method	Dietary exclusion	Main nutritional outcomes
Berry et al. (2015) Finland	Mean age 16 months (range 6-42)	46	3 day food diary	18 milk exclusion 28 excluding milk and wheat	-Markers of nutritional status, nutrient intake and growth were comparable between groups. Mean for anthropometric measures were below average for age in both groups.
Sicherer et al (2012) USA	Mean 23 months (range 6 months to 17 years)	29	Duration of food record not stated	29 CMA of which 27 had multiple food allergies	-At entry to study, the formula was supplying a mean of 61% ± 5% of total calories and 61% ± 7% of total protein compared with 64% ± 4% and 63% ± 4% at 4 months, respectively.
Mabin et al. (1995) UK	Mean 1.8 years (0.6-8.9 years)	45	6 day weighed food record	All 45 consumed a few foods diet (24 casein formula, 21 whey formula)	-Inadequate protein and energy intake in both groups despite consuming a hydrolysate formula. -Median daily volume of casein formula was 10.5ml/day versus 267ml whey formula/day.
Meyer et al. (2014) UK	Median 21 months (range 4 weeks to 16 years)	141	3 day food diary	11 cows' milk only 24 excluded two foods 18 three foods 37 four or more foods	-More children achieved micronutrient requirements if a hydrolysed formula was consumed. -Number of foods eliminated did not impact significantly on micronutrient intake -Boys have greater intake of zinc, calcium, copper, selenium and riboflavin.
Devlin et al. (1989) UK	Range 8 months to 14 years	56	5 day food diary	10 cows' milk containing diet, but excluding other foods. 26 multiple exclusion diet with substitute formula 20 multiple exclusion diet without substitute formula	-75% of those in the multiple exclusion group without substitute formula did not meet calcium requirements. -11% of those in the multiple exclusion group with substitute formula did not meet calcium requirements -No other nutritional information provided.
Madsen et al. (1997) USA	Mean age 9.9 years (range 5-16 years)	58	Food frequency questionnaire assessing calcium intake	58 children with "loosely defined" milk allergy (5 complete milk exclusion, 31 does not drink milk, but eats dairy foods, 16 occasionally drinks milk, 6 drinks milk normally)	53% had calcium intake less than recommended intake. 44% of those who rated their calcium intake fair or good did not meet their requirements. 21 % of those taking supplements still did not meet their requirements. 8% of those who did not drink milk compared to 68% of those who did drink at least some milk met their RDA without supplementation.

2.11.1 Importance of substitute formula

Many of the studies listed in tables 2.5 and 2.6 specifically highlight the important contribution of substitute infant formula to both macro and micronutrient intake (Christie et al., 2002; Devlin et al., 1989; Mabin et al., 1995; Meyer, Koker, et al., 2014; Sicherer et al., 2001; Tiainen, 1995). Although it must be highlighted that some of these studies were funded by infant formula companies so are potentially subject to bias. Sicherer et al's study (2001) (n =29) concluded that consumption of adequate AAF was critical to achieving recommended energy intake for most children. However, the majority of children in this study had multiple food allergies and included participants up to 17 years old, therefore intakes are not necessarily reflective of infants with mild to moderate CMA. Mabin et al. (1995) reported poor intake of casein compared to whey hydrolysate formula, also highlighting the importance of consuming adequate volumes of substitute formula in a few foods diet.

Interestingly, previous research has suggested that the relatively higher protein content of hypoallergenic formula causes infants to consume a lower volume to satiation, which has a potential impact on appetite cues and hunger mechanisms (Mennella, Ventura & Beauchamp, 2011). Although the difference is not large per 100mls (0.5g), in younger infants when total intake can be approximately 1000mls/day, this difference could equate to as much as 5g of protein per day. Both Tiainen et al. (1995) and Henriksen et al. (2002) reported that significantly lower volumes of hypoallergenic formula were consumed by the exclusion group than cows' milk consumed by control groups, however the number of participants was small in both studies so the findings may not be generalisable.

2.11.2 Micronutrients and nutritional supplements

Although inadequate intakes of zinc, selenium and riboflavin have been reported in children consuming exclusion diets (see tables 2.5 and 2.6); calcium and vitamin D are of particular focus in these studies because dairy products are prominent sources. Research in older children with CMA has reported calcium consumption of only 25% of recommended intake, resulting in reduced bone mineral status (Jensen, Jorgensen, Rasmussen, Molgaard, & Prahl, 2004). Likewise Madsen et al. (1997) reported that 53% of children had calcium intake below requirements and 44% of those who rated their calcium intake 'fair' or 'good' did not meet their requirements. This study included some subgroups who consumed small amounts of dairy products, illustrating that calcium intake can be suboptimal even with partial CME (Madsen & Henderson, 1997).

Although supplementation with both calcium and vitamin D is common, studies disagree

whether supplementation is adequate to meet requirements. In a French study of 28 children with CMA (Flammarion et al., 2011), half of participants received a calcium supplement. Although calcium intake derived from food was lower than for the control group, overall intake met recommended levels with the addition of a supplement. In contrast, none of the participants in Henriksen et al's study (2000) were achieving their calcium requirements, despite nearly half of the children taking regular calcium supplements. This emphasises the importance of establishing adequate calcium intake from substitute foods early on. Meyer et al.'s study (2015), which included children consuming both single and multiple exclusion diets, reported that 10% of those with and 60% of those without a vitamin supplement had sub-optimal vitamin D intakes, underlining the importance of individualised advice.

2.11.3 Growth of children consuming cows' milk exclusion diets

As is the case with studies of nutritional intake, studies investigating growth parameters in children consuming exclusion diets are heterogenous in study design, food groups excluded, population, methods and setting. Therefore for clarity and brevity, this section will refer only to growth studies of children consuming substitute formula or CME diets, rather than discuss growth studies of children consuming exclusion diets for food allergy in general or the effect of eczema on growth. Growth will be further explored in the context of other food exclusion diets in later chapters.

The mechanism for impaired growth in children with CMA is attributed to dietary restriction and/or the underlying pathophysiology of the allergic disorder, including inability to utilise nutrients due to chronic inflammation (Vieira et al, 2010). A study of children under two years old with suspected CMA in Brazil reported that nutritional deficits were commonplace with 15.1%, 8.7% and 23.9% having low weight for age (underweight), low weight for height (wasting) and low height for age (stunting) scores respectively. However these were children seen at first evaluation at a paediatric gastroenterology clinic, many had been given inappropriate substitute formula and no data is provided on time lag between onset of symptoms and attendance at clinic. There was also no differentiation made between IgE and non-IgE CMA, diagnosis was not challenge-proven and although there is a relatively large sample size of 159 children, the data is cross sectional.

Studies of infants with challenge proven CMA have also reported impaired growth (Agostoni et al., 2007; Isolauri, Sutas, Salo, Isosomppi, & Kaila, 1998). Agostini et al. (2007) reported that infants with CMA who were breastfed for at least four months have low weight for age and length for age at six months. In a Finnish study of 100 infants with CMA, relative

weight and length fell compared to the control group, with no catch up seen by two years (Isolauri et al., 1998). Delay in growth was more pronounced in the subgroup who had symptoms before six months old. Notably, a disproportionate amount of nutrients derived from the substitute formula at the expense of introduction of solid foods contributed to poor growth. Poor growth was attributed mainly to low-grade antigen exposure and chronic skin and gut inflammation.

In contrast, a second Finnish study of infants with challenge proven CMA (n = 168) indicated that weight for length was just under the 50th centile at age two and four years. There was no difference between EH whey and soya formula groups and no difference between those with IgE or non-IgE mediated allergy (Seppo et al., 2005). Although this study was longitudinal, it did not include a control group or a group fed an AAF. Participants received individualised dietetic counselling with 70% of children still consuming a substitute formula at age two years, which may explain the good growth achieved.

A small number of studies have investigated the use of substitute formula in either high risk or healthy infants, rather than infants with CMA (Mennella, Ventura, & Beauchamp, 2011; Rzehak et al., 2011). This type of study design therefore allows exploration of the effect of the formula per se, without the effect of the allergic condition. In a large prospective German study (n = 1386), infants at high risk of atopy were randomised to one of three EH formula groups or a cows' milk formula group for a period of sixteen weeks (Rzehak et al., 2009). Infants fed an EH casein formula showed slower weight gain in the first 48 weeks of life, compared to infants fed EH whey formula, breast milk or cows' milk formula. However the lower weight gain in the EH casein formula group was transient, with no difference seen between groups at any other stage up until ten years of age (Rzehak et al., 2011). One explanation for this was that the lower palatability of the EH casein formula resulted in lower intake and consequently in a retarded weight gain in the first year of life. A smaller study of healthy infants has reported similar findings, showing that those fed a cows' milk formula have accelerated weight gain in the first seven months of life compared to infants fed an EH casein formula, who had normal growth (n= 56) (Mennella et al., 2011). This finding may be of significance as data from healthy infants indicate that early nutrition may affect health in later life, particularly in infants with rapid growth acceleration (Singhal, 2013).

2.12 Unnecessary exclusion of cows' milk

Because cows' milk is the main source of nutrition for infants (either via breast milk or through infant formula), it is therefore not surprising that it is often identified as a possible cause for

skin and gut problems. Research suggests that nearly 50% of parents will change their infant's formula in the first six months of life, usually without consultation with a health professional, and often for a perceived health problem (Nevo, Rubin, Tamir, Levine, & Shaoul, 2007). The most common reasons for formula change were regurgitation, vomiting, and fussiness, indicating that parents perceived that these symptoms reflect intolerance to formula. Adverse reactions are commonly falsely attributed to cows' milk, meaning exclusion diets are sometimes initiated unnecessarily (Eggesbo, Botten & Stigum, 2001; Sinagra et al., 2007). Although challenge-proven CMA is estimated to affect less than three per cent of infants in the UK, it is known that parents may incorrectly perceive their child to have a food allergy (Venter et al., 2006). In a Danish birth cohort study, although 6.7% of children were perceived to have symptoms of CMA, only 2.2% were proven to have CMA on challenge (Høst et al., 2002). Likewise in a UK birth cohort study, only one third of those eligible for food challenge based on history and SPT had challenge proven CMA (Schoemaker et al., 2015). In the UK, where allergy services are considered to be inadequate for demand (Royal College of Physicians, 2010) the problem of unsupervised exclusion diets is heightened, meaning infants may remain on an unnecessarily restrictive diet for a prolonged length of time before they are assessed by an allergy specialist.

The problem of misperceived CMA is such that it has been claimed "mislabelling non-allergic infants as being allergic to cow's milk is more common than CMA itself" (Elizur, Cohen, Goldberg, Rajuan, & Katz, 2013). In a large population based cohort study in Israel (n=13019), 1.9% of infants were said to have been misdiagnosed with CMA. This subgroup of infants was more likely to present within the first three months of life with symptoms involving a single organ. Higher parental education level was associated with mislabeled reactions, specifically higher maternal education was associated with non-specific symptoms such as 'restlessness'.

Similarly, a prospective cohort study in Switzerland (Bergmann et al., 2014) reported that the large majority of infants under six months old who have short lasting, benign symptoms do not meet the diagnostic criteria for CMA. The authors concluded that the diagnosis of CMA and subsequent implementation of an exclusion diet should only be considered in a minority of infants with persistent and severe symptoms. However it must be noted that this study did not recruit infants who were exclusively breastfed, which may bias the findings. A Dutch study found that of the 7% of children who visit their GP for suspected CMA every year, only 56% underwent a food challenge (van den Hoogen et al., 2014). Despite none having confirmed CMA, a long-term milk substitute formula was prescribed in 71% of cases. Overall these three studies demonstrate that misdiagnosis of CMA is a widespread issue that occurs in many

countries.

2.13 Natural history and resolution of cows' milk allergy

2.13.1 Assessing resolution of cows' milk allergy

The resolution of CMA is assessed differently depending on the underlying immunological mechanism. For IgE-mediated CMA, tolerance is assessed by allergy testing to detect a decrease in either SPT wheal size or specific IgE level. A hospital based challenge is usually arranged when tolerance is suspected. In non-IgE mediated CMA, tolerance is usually assessed using a gradual home introduction plan, although this should only be undertaken in selected infants with mild or moderate non IgE CMA (Venter et al., 2013). An example of a home reintroduction plan for mild to moderate non-IgE CMA is shown in Figure 2.3 (Venter et al., 2013). Reintroduction starts with baked milk and progresses in quantity and allergenicity through a number of stages with the final step being introduction of fresh (uncooked) milk.

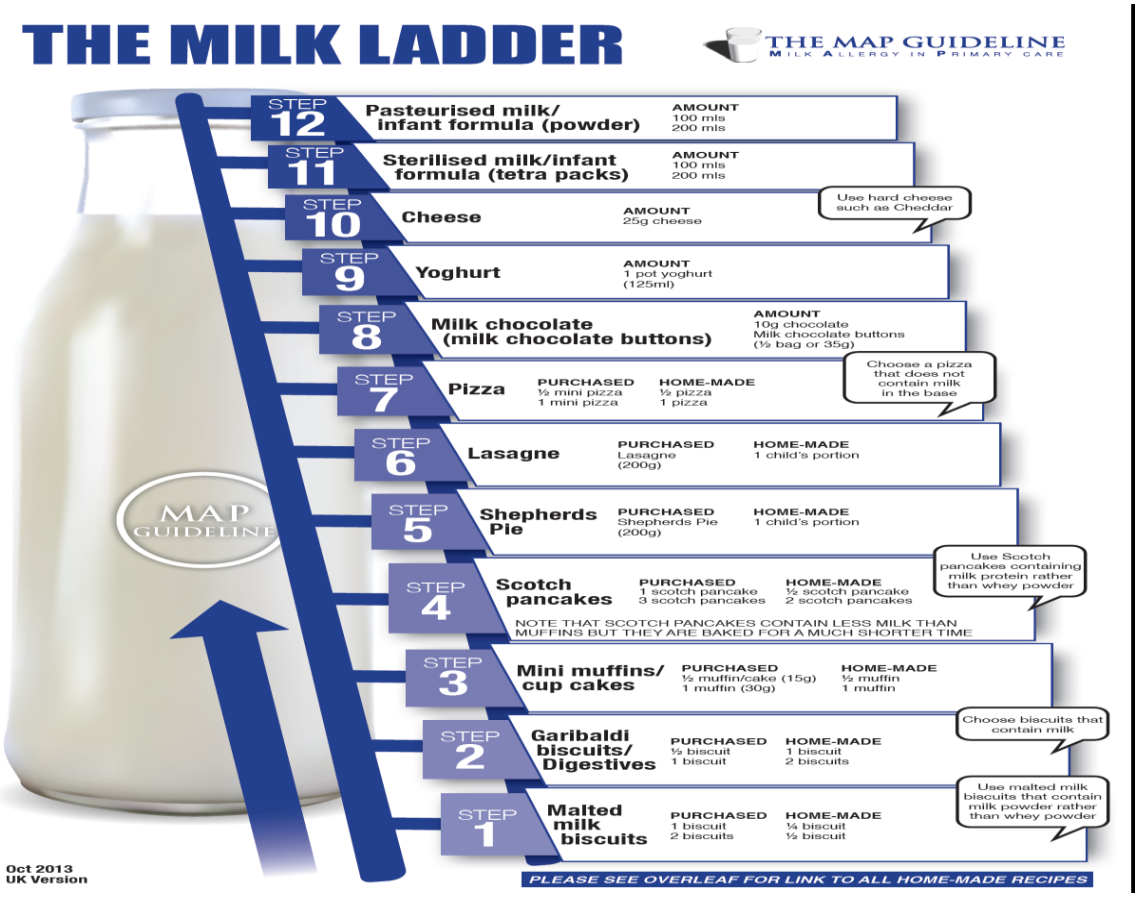

Figure 2.3 Cows' milk reintroduction plan for mild to moderate non-IgE CMA

2.13.2 Natural history of cows' milk allergy

The natural history of CMA has been extensively investigated by a number of studies from different populations around the world. Differences in selection criteria of the study population, diagnostic criteria, age at follow up and type of CMA mean that results are not entirely conclusive regarding the age at which resolution occurs. However, generally speaking there is a consensus that CMA is a condition of childhood and typically those with non-IgE mediated CMA will acquire tolerance earlier than those with IgE-mediated CMA (Høst et al., 2002; Saarinen, Pelkonen, Mäkelä, & Savilahti, 2005; Santos, Dias, & Pinheiro, 2010; Schoemaker et al., 2015). Most studies report that the majority of CMA will resolve by age 2-3 years old, although severe phenotypes exist which may persist into older childhood and adolescence.

A Danish birth cohort study (Host et al., 1995) is commonly cited regarding the natural history of CMA. A good prognosis of CMA was reported, with acquisition of tolerance to cows' milk in 56% of children by age one year, 77% by age two years, 87% of children by the age three years, 92% by age five years and 97% at age 15 years. In contrast, some studies published since then report an older age of acquisition of tolerance. In another large birth cohort study (Saarinen et al., 2005), it was reported that 15% of participants with IgE-mediated CMA had persisting disease at eight years of age, yet all of those with non-IgE mediated CMA had outgrown it by age five years. Likewise a study of 155 children with IgE-mediated CMA from a tertiary hospital setting in the United States reported that only 42% had acquired tolerance at eight years of age (Skripak, Matsui, Mudd, & Wood, 2007).

A Portuguese study of children with CMA selected from a clinic population reported only a quarter acquired tolerance before the age of two years, however data was collected retrospectively and some participants lost to follow up (Santos et al., 2010). Immediate allergic symptoms, asthma and other food allergies were independent factors for the persistence of CMA beyond the age of two (Santos et al., 2010). A study investigating the natural history of CMA in nine countries across Europe reported that 69% tolerated cows' milk one year after diagnosis, which included all of the children with non-IgE mediated CMA and just over half of those with IgE-mediated CMA. However only 58% of those who were diagnosed with CMA were re-evaluated, with variations between countries from 0% to 80%. In the UK arm of the study, of the eleven children with challenge proven CMA, seven were re evaluated after one year and five were tolerant (Schoemaker et al., 2015).

2.13.3 Incomplete resolution of cows' milk allergy

Although the vast majority of infants and children with CMA will outgrow the condition, the

literature suggestions there is a subgroup of individuals who experience incomplete resolution of CMA with symptoms persisting at a sub clinical level for several years. This is thought to be a “residual intestinal disease” (Kokkonen, Tikkanen, & Savilahti, 2001) and that not all children will consume a normal intake of dairy products as a result (Dupont, 2013).

Kokkonen et al. (2001) undertook a study of Finnish children, aged 9–11 years, investigating whether children with previous CMA have a complete recovery of gastrointestinal symptoms. All children in the study group (n = 56) had been consuming a CME diet until at least two years of age, after which they had negative food challenges. They were compared to aged matched healthy control children. Approximately half the study subjects (45%) reported milk-related gastrointestinal symptoms, compared to 10% of control participants. Those who reported symptoms underwent a four week blind elimination-challenge test, with three of six study subjects and seven of ten control subjects responding with intestinal symptoms. Interestingly, the growth of the former CMA participants was significantly lower compared with the control subjects, and the difference in height was most obvious in those subjects still reporting milk-related gastrointestinal symptoms. Lactose malabsorption was found in eight CMA subjects (14%) and six control subjects (3%). The authors concluded that a proportion of children with CMA in infancy have persistent, but mild and vague, gastrointestinal symptoms. However, they did not measure dietary intake to determine whether this affected food consumption.

More recently, a similar study from the USA identified a higher frequency of gastrointestinal symptoms and functional gastrointestinal disorders in children previously diagnosed with CMA compared to healthy controls (Saps, Lu, & Bonilla, 2011). Children aged 4-18 years old who were diagnosed with CMA in infancy were matched to healthy siblings without a history of CMA. The study determined that 44.2% of children with a history of CMA reported gastrointestinal symptoms including abdominal pain, constipation, or diarrhea compared with 20.75% of controls. Additionally, nineteen percent of the CMA group compared to none of the control group was diagnosed with a functional gastrointestinal disorder (mostly irritable bowel syndrome) using strict diagnostic criteria. The authors hypothesised that early-life inflammation caused by CMA can cause long-term changes in the brain-gut axis leading to altered pain pathways and persistent visceral hyperalgesia. They also suggest that use of hypoallergenic formulas for treatment of CMA may be futile for preventing long term gastrointestinal symptoms, if there has already been several weeks of gut inflammation by the time treatment is commenced. Although the findings are very thought provoking, the study’s use of retrospective design is highly subject to recall bias, in addition to the fact that CMA was

not diagnosed using an objective challenge procedure. Furthermore dietary intake was not measured.

2.14 Summary of literature review on cows' milk allergy

CMA is the most common infant food allergy, but has a good rate of resolution, usually by 1-2 years of age. It may present with a spectrum of symptoms presenting in the skin, respiratory and gastrointestinal systems. Symptoms may be immediate, delayed or chronic. It is managed by strict exclusion of cows' milk, use of substitute infant formula and/or maternal exclusion diet. Substitute infant formulas used in the management of CMA have a distinctive and unpalatable taste.

Dietary exclusion of cows' milk should ideally be supervised by a dietitian with training in food allergy. Exclusion of cows' milk can led to nutritional deficiencies and impaired growth in some situations, although the evidence is not consistent. Perception of CMA is higher than proven CMA, therefore unnecessary and unsupervised dietary exclusion does occur. Reintroduction of cows' milk usually takes place with a graded approach, although preliminary evidence suggests incomplete resolution of CMA can occur in a minority of cases, with some children and their parents reluctant to reintroduce all forms of cows' milk into the diet.

3 Chapter three: Literature review of infant and childhood eating behaviours

3.1 Overview of chapter

This chapter considers the development of infant and childhood eating behaviours. It begins with an examination of infant taste development and the role of early exposure in the evolution of food preferences and dietary variety. It will then explore the topics of fussy eating, feeding difficulties and food neophobia in toddlers and the longitudinal tracking of these behaviours into later childhood and early adolescence. Throughout this chapter, reference will be made to the interaction of CMA with feeding behaviours, where research exists.

3.2 Introduction

It is broadly accepted that children eat the foods they like and they like the foods that they are familiar with (Birch, 1999; Cooke & Fildes, 2011; Scaglioni, Arrizza, Vecchi, & Tedeschi, 2011). The flavour of a food determines its acceptability and modulates intake (Beauchamp & Mennella, 2011). This effect is more evident in children than adults, because children are less influenced by cognitive and experiential factors (Birch, 1979). The pattern of flavours that an infant is exposed to contributes to their unique pattern of food preferences. This is important from a public health perspective as excess intake of salty and sweet foods is related to many long-term conditions. Therefore the early origins of disease may derive from taste and food preferences that are "imprinted" from infancy (Beauchamp & Mennella, 2011; Birch & Doub, 2014; Scaglioni et al., 2011). Figure 3.1, adapted from Daniels et al. (2009) provides an overview of the factors that influence eating behaviour in children, many of which will be addressed during the rest of this chapter.

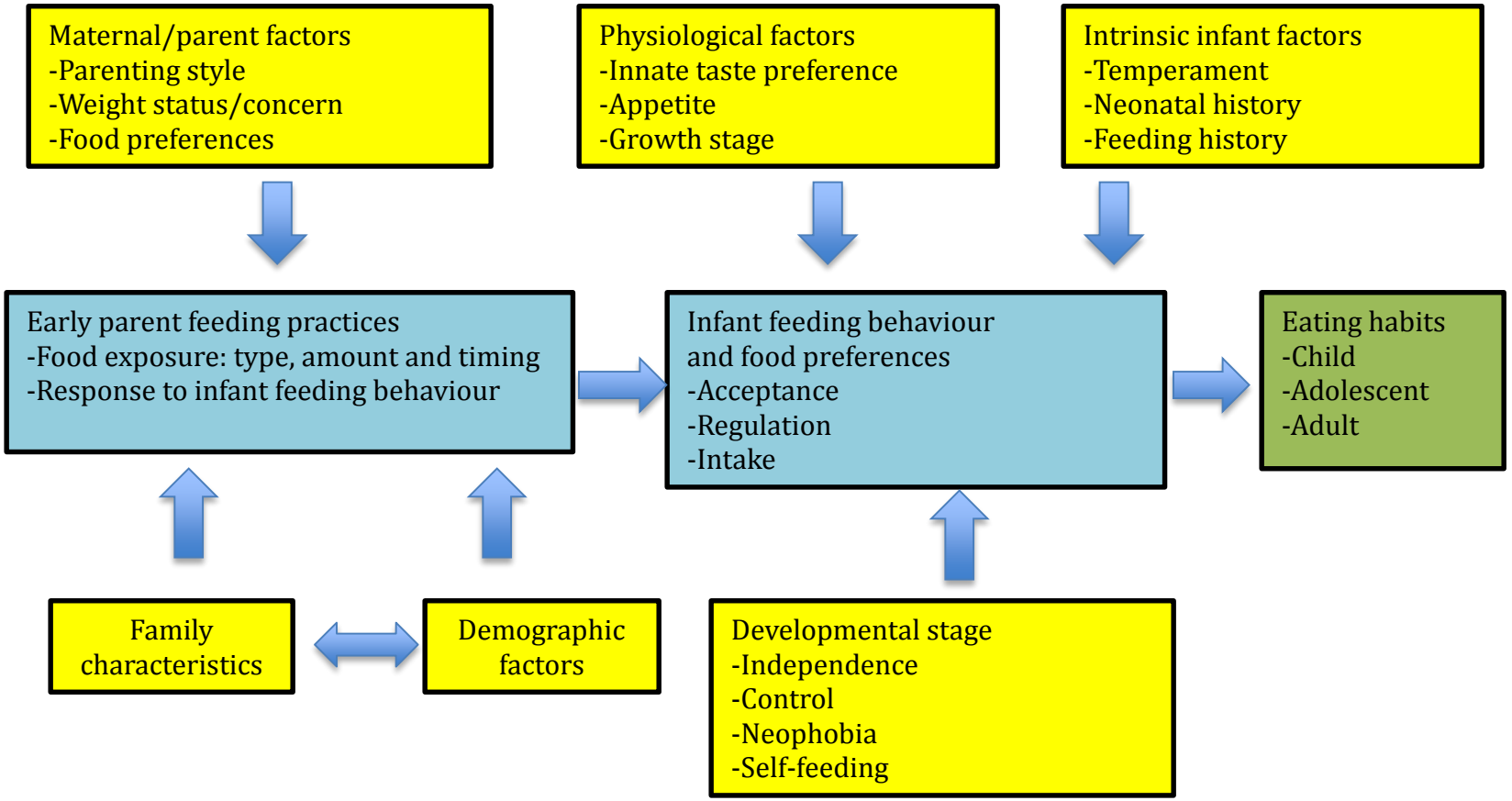

Figure 3.1 Factors that influence the development of infant and child eating habits

3.3 Physiology of taste and flavour preference

The flavour of a food or drink is composed of three distinct components: taste, odour and chemical irritation. These three components have separate anatomical systems, but interact to give a unified sensory experience (Beauchamp & Mennella, 2011). Behavioural responses to food and taste are choreographed by the integration of gustatory information, however the perception of taste in humans is also modulated by hunger, satiety, emotion and expectation (Yarmolinsky, Zuker, & Ryba, 2009).

The sensation of taste occurs when specialised cells called taste receptors are stimulated. Taste receptors consist of modified epithelial cells, arranged into groups of 50-150 receptors to form clusters, commonly known as taste buds. Taste buds are abundant on the tongue, but are located throughout the oral cavity (on the hard and soft palates, the pharynx, larynx, tonsils, oesophagus and epiglottis) (Yarmolinsky et al., 2009). Taste buds are organised into structures called papillae, which are innervated by branches of three cranial nerves: facial nerve (VII), glossopharyngeal (IX) and vagal (X) nerves (Negri et al., 2012). The physiology of taste is illustrated in Figure 3.2 (image sourced from www.brainfacts.org)

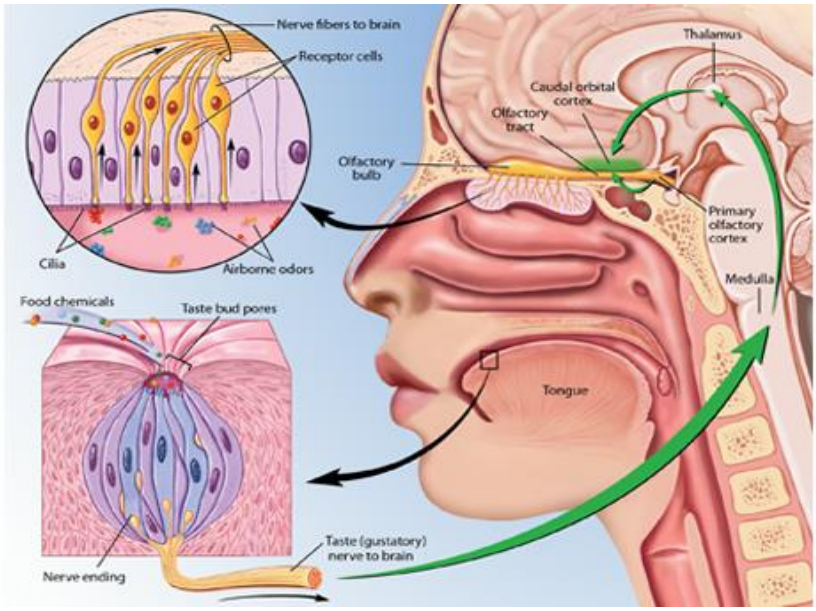

Figure 3.2 The physiology of taste

There are five basic tastes: sweet, salty, sour, bitter and umami (savoury). There is also a suggestion that a sixth basic taste of fat may exist, however five primary tastes are generally referred to (Running, Craig, & Mattes, 2015). All five basic tastes can be perceived in all areas

of the tongue. Taste receptors may also be located in the gut and it is thought that the taste system extends along the digestive tract (Beauchamp & Mennella, 2009; Yarmolinsky et al., 2009). Sweet, bitter and umami tasting molecules bind to specific taste receptors and transmit information from the taste buds to specific areas of the brain where the taste is decoded and judged. Sour and salty tastes are mediated by ion channels located on the taste cell, rather than binding of molecules to receptors, however the exact mechanisms are unknown (Beauchamp & Mennella, 2009; Yarmolinsky et al., 2009).

3.4 Innate taste preferences

Newborn infants are known to be responsive to different taste stimuli. Sense of taste has evolved to discriminate nutritive foods from potential poisons. Taste buds are said to be gatekeepers whereby they protect humans from ingesting harmful items and form the decision point on whether to accept or reject foods (Yarmolinsky et al., 2009). Generally, a sweet taste evokes a positive reaction, whereas both sour and bitter tastes provoke negative reactions (Beauchamp & Mennella, 2009). Sweet and umami tastes signal a source of calories from carbohydrates and proteins respectively.

Unpleasant tastes (i.e. bitter and sour) signal the presence of toxins or acids (Birch, 1999; Yarmolinsky et al., 2009). A salty taste may be perceived positively or negatively depending on the concentration (Negri et al., 2012; Yarmolinsky et al., 2009), with identification and preference for salt thought to develop at approximately four months of age (Mennella, Ventura, & Beauchamp, 2011). Despite the fact that these preferences are inbuilt, they can be modified through exposure in utero, during early infancy, in childhood and in adolescence (Birch, 1999). As an example, tolerance to bitter tastes changes with age, demonstrated by people learning to like bitter tasting foods and drinks (e.g. coffee). There is also a genetic predisposition to certain taste preferences, which will now be explained.

3.5 Genetic perception of taste

There is a continuum whereby individuals vary in their ability to detect bitter tastes dependent on their genotype (Golding et al., 2009). There are thought to be at least twenty genes involved in the ability to detect bitter taste, the most important gene being TAS2R38 (Behrens & Meyerhof, 2006). Approximately one quarter of the Caucasian population find bitter tastes extremely unpleasant and are known as “supertasters” (Wardle & Cooke, 2008). On the other end of the continuum approximately a quarter of individuals detect no taste or a very mild taste (“non-tasters”). Approximately half can detect a bitter taste, but are relatively neutral to it, known

as “medium tasters” (Golding et al., 2009). The influence of genotype may attenuate with age and exposure as there is known to be a higher prevalence of supertasters amongst children than adults (Negri et al., 2012).

In practical terms, this may mean that supertasters are less likely to enjoy and consume bitter foods, such as green cruciferous vegetables. It is also thought that the TAS2R38 gene may directly or indirectly influence perception of sweetness (Keller et al., 2014; Mennella, Pepino, & Reed, 2005) and/or other strong flavours. This may be due to bitter receptors indirectly binding sweet tastes or may be because sweetness is used to mask a bitter taste and preference is developed over time.

Overall the evidence base is inconsistent and there is a lack of longitudinal studies. A study of 4-5 year old children (Keller, Steinmann, Nurse, & Tepper, 2002) reported that acceptability of some bitter and high fat foods may be in part genetically determined; finding differences in liking for broccoli, cheese and full fat milk between supertasters and non-tasters, although differences were not found for other foods such as orange juice, chocolate and skimmed milk. Results of a food frequency questionnaire (FFQ) found no difference in the reported consumption of sweet and fatty foods, suggesting that taster status did not translate to altered dietary intake. Similarly, a study of 3-6 year old children found that bitter tasters disliked raw spinach, but there was no correlation with liking for raw or cooked broccoli, banana, lemonade, cheese or whole milk (Turnbull and Matisoo-Smith, 2002). The authors acknowledged that there might be limitations in conducting sensory studies in children of that age.

In older children, the results also suggest that bitter taster status has a subtle, but unconvincing effect. A study of children aged 5-10 years (n = 143) and their parents, found that children with alleles of the bitter tasting gene preferred higher concentrations of sucrose, breakfast cereals and beverages with a higher sugar content. This effect was not consistent across different ethnicities and was not present in adults, suggesting cultural and experience factors may override the effect of the genotype (Mennella et al., 2005). A large study using a UK birth cohort (n = 5294) reported that tasters were more likely to be described by parents as “fussy eaters” than non tasters at age ten years, however there was no dietary assessment conducted to validate this finding (Golding et al., 2009). An Irish study of 7-12 year old children (n = 525) reported twenty per cent of variation in food preference could be explained by taster status, concluding that a genetic predisposition to bitter sensitivity was outweighed by environmental influences.

In terms of growth, studies investigating the aetiology of obesity have examined the effect of taster status on weight. No difference in body mass index (BMI) was found between taster groups at age 4-6 years (Keller et al., 2014) or ten years (Golding et al., 2009). Overall it can be said that genetic variation in taste perception exists, particularly to sweet and bitter tastes, although it is thought to only have limited influence on food preferences in daily life (Wardle & Cooke, 2008) and is unproven to have an effect on weight status. Genetics of taste preference will not be measured in this thesis, however an awareness is important in order to provide a contextual background.

3.6 In utero taste exposure

Although innate preferences exist, there is individual variation in infants' taste preferences depending on the tastes and odours present in the prenatal environment. Characteristics of the maternal diet are transmitted to the infant via amniotic fluid and later via breast milk, therefore providing the infant with an early chemosensory experience (Cooke & Fildes, 2011). A systematic review of the effect of prenatal and early infant taste experiences on later taste acceptance concluded there is a clear programming effect for bitter taste, but studies on sweet and salty were equivocal. Twenty studies were included in the review, but there were limited studies on sour and umami tastes (Nehring et al., 2015).

The first experimental study to demonstrate the in utero effect randomly assigned pregnant women to one of three groups (Mennella, Jagnow, & Beauchamp, 2001). Participants consumed either 300 ml carrot juice or water during the last trimester of pregnancy and the first two months of lactation. Group one drank carrot juice during pregnancy and water during lactation; group two did vice versa. A control group drank water during both pregnancy and lactation. Infants consumed a test meal of carrot-flavoured cereal at the beginning of complementary feeding. The results demonstrated that previously exposed infants exhibited fewer negative facial expressions while feeding the carrot-flavoured cereal compared to a plain cereal and had a tendency to eat more than the control group. The methods used in this study were relatively subjective, however the results were not influenced by maternal eating habits or attitudes to food. Although this study was simple in design, only examining the effect of one food in a small sample size, it is known that a wide variety of flavours (e.g. fruit, vegetables, spices) are transmitted via the amniotic fluid (Mennella 2014).

3.7 Exposure to tastes via breastmilk

Whilst amniotic fluid provides an initial exposure to flavours, breastfeeding is said to act as a "flavour bridge" between exposures in utero and solid food (Mennella & Trabulsi, 2012). This is illustrated in Figure 3.3, adapted from Mennella & Trabulsi (2012). It is thought the variation in oral sensory experience that a breastfed infant is exposed to prepares them for novel tastes when solid foods are introduced. This is in contrast to infant formula milk, which is uniform in taste, meaning the infant is constantly exposed to a single flavour (Cooke & Fildes, 2011).

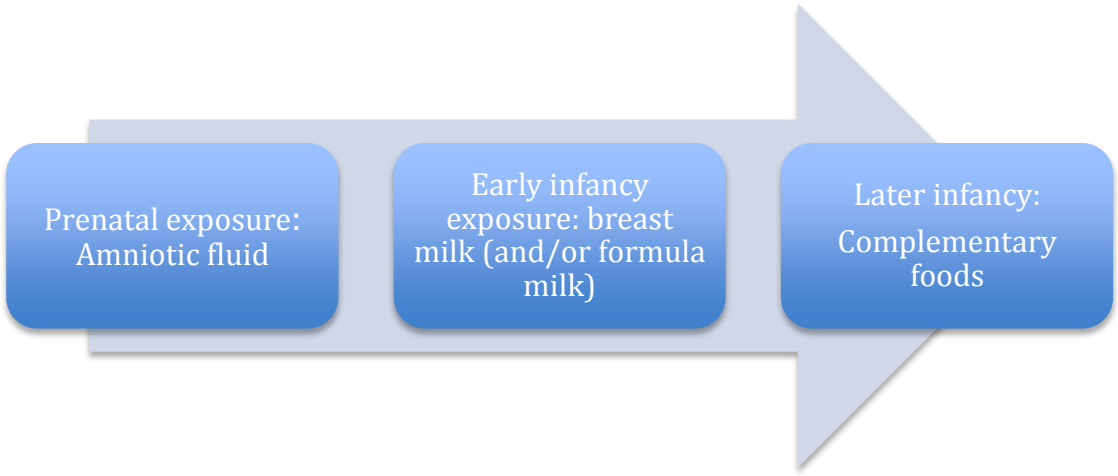

Figure 3.3 Continuum of exposure opportunities to early tastes.

To illustrate the effect of breastfeeding on exposure to flavours, a study of 5-7 month old infants (n = 142) from two European countries investigated differences in acceptance of new foods introduced during the complementary feeding process (Maier, Chabanet, Schaal, Leathwood, & Issanchou, 2008). Although there was no difference in acceptance of the first food offered (carrot), breastfed infants subsequently ate more of four unfamiliar foods offered (tomato, peas, fish and meat) than formula fed infants. The effect persisted for some foods when re tested two months later, but the long-term effect was not evaluated. As with all studies involving infant feeding, a limitation of the study was that it is not possible ethically to randomise against breastfeeding.

Another study comparing breast to formula fed infants (n = 45) found that breastfeeding confers an advantage in initial acceptance of a food, but only in mothers who eat the food or similar foods regularly (Forestell & Mennella, 2007). However it is not known how often mothers need to eat specific foods during lactation to increase their child's liking of it (Forestell & Mennella, 2007). A large Australian study reported a modest association of breastfeeding duration and food variety at age two years, independent of maternal demographic characteristics (Scott, Chih, & Oddy, 2012), however dietary assessment was limited to one

single 24 hour recall of intake. Looking longer term, a study of children aged 6-8 years old using three days of dietary data found a modest association between breastfeeding duration and fruit variety consumed, but no association with vegetable variety consumed (Skinner, Carruth, Bounds, Ziegler, & Reidy, 2002).

A limitation of studies of this nature is the high rate of attrition in exclusive breastfeeding. In a study investigating the association between duration of breastfeeding and infant food preference (n = 122 mother and infant dyads), only 16% of infants were exclusively breastfed for at least six months (Schwartz, Chabanet, Laval, Issanchou, & Nicklaus, 2014). An association between duration of exclusive breastfeeding and preference for umami taste was reported at six months, which was hypothesised to be due to the higher levels of glutamate in breast milk than formula milk. No difference was found between duration of exclusive breastfeeding and sweet, salty, sour and bitter taste acceptance at six or twelve months. However, this study did not measure maternal or infant diet, which are confounding variables. The measurement of infant taste preference and acceptance is often reliant on subjective measurements, either analysis of facial expressions or mothers' judgment. A strength of this study was the use of an objective measure of ingestion ratio (i.e. volume of umami solution consumed relative to plain water consumed).

3.8 "Imprinting" of tastes via formula feeding

Studies investigating the effect of formula feeding on taste preferences are plentiful, however most have investigated the effect of specialised infant formula used in specific disease conditions, which have an altered taste, rather than a standard infant formula (Liem & Mennella, 2002; Mennella & Beauchamp, 2002; Mennella, Forestell, Morgan, & Beauchamp, 2009; Owada, Aoki, & Kitagawa, 2000; Sausenthaler et al., 2010). Milk, whether formula or breast milk, is the first infant food and becomes the standard against which all other new foods and flavours are evaluated (Birch & Doub, 2014). This is particularly salient when the milk that is fed has an altered or unusual flavour.

It is said that the characteristic flavour of a formula is "imprinted" from an early age and remains a preference for some time (Owada et al., 2000). This was demonstrated by a study which trialled a new low peptide feed for management of phenylketonuria (PKU) against an existing AAF. In children under 18 months old, 90% of those with PKU (who were accustomed to the taste of the AAF) compared to 66% of healthy children (who had never been exposed to the taste of a specialised formula) accepted both formulas. In children aged between 18 months and 11 years, a quarter of the control group strongly disliked both formulas, compared to none

of the PKU group. This study therefore demonstrates an effect of age in acceptance of specialised formula, suggesting the younger children in the control group were more accepting of the unpalatable specialised formula than older children. However this was a cross sectional study with a relatively small overall sample size (n = 88) and the taste acceptance in children under five years old was measured by parental or doctor evaluation of facial expression, rather than using an objective observer or measurement of intake.

The area where this has been most thoroughly investigated has been with substitute infant formulas designed for CMA. The majority of these studies have been conducted by the Monell Sensory Science group in Philadelphia, USA. These studies are summarised in Table 3.4. However before discussing these studies, it must be emphasised that they generally did not recruit infants with proven CMA, instead healthy infants were usually randomised to either a control group or a substitute formula group as part of the study design. One of the studies included infants who were prescribed the formula due to suspected allergy (Mennella 2005), but no information is provided about the allergy or symptom history. Another weakness of these studies is that limited or no data is reported about the timing or type of complementary foods consumed by the participants, which is a major confounding factor. Additionally, EH casein and soya formula are the only substitute formula types used in these studies; there are no AAF or EH whey formula groups. Therefore although these studies provide very insightful data into the sensory effect of some substitute formulas on taste preference, there are major limitations. Not least the fact that the substitute formulas were generally not used for therapeutic indications, therefore the influence of allergic symptoms and disease cannot be evaluated.

Table 3.1 Summary of studies investigating the effect of extensively hydolysed formula on taste preferences

Authors and year	Number & age of participants	Groups	Methods	Results
Mennella Griffin & Beauchamp (2004)	n = 53 aged 7.5 months	1.Cows' milk formula 2.EHF casein (Nutramigen) 7 months. 3.EHF casein (Nutramigen) for first 3 months only 4. EHF casein: Nutramigen from months 2-5.	-Monthly test feeds to assigned formula. Test feed to cows' milk formula, Nutramigen and Alimentum (novel formula) on 3 separate days age 7.5 months. Outcomes: Volume & length of feed, maternal rating of enjoyment and video analysis.	-Exposure to EHF for 7 months showed greater acceptance to EHF than exposure for only 3 months -Infants in CMF group strongly rejected EHF at age 7.5mo. -No difference between groups 3 and 4.
Mennella & Beauchamp (2005)	n =49 aged 5-11 months	1. Cows' milk formula (control group) 2. EHF casein: Nutramigen 3. EHF casein: Alimentum	Infants given test feed of both EHF casein brands. Video analysis, length of feed and volume recorded.	-Milk fed infants rejected both EHFs equally. -EHF fed infants preferred the brand they were being fed.
Mennella, Forestell, Morgan & Beauchamp (2009)	n = 97 Aged 4-9 months	Three groups of infants: recruited retrospectively: 1. Cows' milk formula 2.EHFcasein 3. Breastfed	-Test meals of plain, sweet, salty, bitter, sour and savoury cereal. -Acceptance measured with volume, length and rate of eating. -Video analysis of facial expression and maternal rating of acceptance. -Food frequency questionnaire	-In infants not yet weaned, EHF casein group ate more savoury, bitter and sour cereal and ate a faster rate than breastfed or cows' milk formula fed. In those who had been weaned, no difference between groups. -If exposed to cheese, ate more salty cereal. If ate broccoli, ate more bitter cereal. If ate pasta/tomato, ate more savoury cereal
Mennella, Lukasewycz, Castor & Beauchamp (2011)	n = 69. Aged 7.5 months	1.Cows' milk formula for 7 months 2. EHF casein for 7 months 3. EHF casein:1 month at 1.5 months 4. EHF casein: 3 months at 1.5 months 5. EHF casein:1 month at 2.5 months 6. EHF casein:1 month at 3.5 months	-Test feed of EHF casein and cows' milk formula each month with measurement of volume and maternal rating of acceptance.At end of trial: test meal of both formula with measurement of volume, maternal rating of acceptance and video analysis.	- Rejection of casein EHF was greatest in those who first tried EHF aged 3.5 months. Those fed EHF for only 1 month were as accepting of the formula as those fed for 3 months, but those fed EHF for 7 months were most accepting.
Mennella & Castor (2012)	n = 46 8.5 months	1. Cows' milk formula 2. EHF casein for 1 month 3. EHF casein for 3 months 4. EHF casein for 8 months	Test meal age 8.5 months 1. Plain vegetable broth 2. Vegetable broth with additional monosodium glutamate (= umami flavor). Outcome measures: volume, maternal rating of enjoyment, video analysis at end of trial.	-Those who were fed EHF for 3 or 8 months ate more and umami broth and at a quicker rate than plain broth, compared to controls. -No difference between control and one month EHF group.

The studies listed in Table 3.1 all differ somewhat in their study design, for example one study explored taste preferences of two different brands of EH casein formula (Mennella & Beauchamp, 2005), indicating that taste preference is brand-specific. Only one study included a breastfed group (Mennella, Forestell, Morgan, & Beauchamp, 2009) and the authors acknowledged that there were differences in the timing and type of weaning foods that the breastfed group consumed, underlying the difficulty in minimising confounding factors and bias in infant feeding studies. This study reported that the EH casein formula group ate more savoury, bitter and sour cereal and ate a faster rate than breastfed or cows' milk formula fed infants. However the effect was not observed in infants who were consuming solids, implying that the effect of the EH casein formula is overridden once flavours from solid food come into play. Other studies have sought to demonstrate whether there is a specific timing and duration of (i.e. a "window of opportunity") when manipulation of taste preference is likely to occur (Mennella, Griffin, & Beauchamp, 2004; Mennella & Castor, 2012), demonstrating that the longer an EH casein formula is consumed, the greater preference for both the formula itself and a savoury broth.

The concept that there is a period of plasticity in postnatal development when there is an ability to change innate preferences based on experience has been reported (Mennella &Trabulsi 2012). Indeed it has been claimed that up to six months of age is a sensitive period for the introduction of varying flavours and that after this point, humans are never again as willing to accept novel tastes (Cooke & Fildes, 2011). In contrast it has been said that learning about foods is a continual process and adults learn to accept new, exotic, sophisticated foods at almost any stage of life (Szczesniak, 2002). Others argue that learning of new flavours can occur across the life span, but greater plasticity and more permanent effects of early compared with later flavour experiences occur (Beauchamp & Mennella, 2011). The aforementioned studies in Table 3.1 do imply and confirm that there is a sensitive period before four months of age and also provide strong evidence of a dosing effect, whereby those who were fed an EH casein formula for seven months were more accepting that those fed it for three months (Mennella et al., 2004).

3.9 Solid food introduction

The introduction of solid food to infants' diets, known as "complementary feeding" (World Health Organization, 2002) is a significant milestone that has nutritional, developmental and health implications. In the UK this process is known as "weaning". In this thesis the two terms will be used interchangeably, even though it is acknowledged that the WHO define "weaning" as

cessation of breastfeeding. Weaning is a necessary transition to meet the changing nutritional and developmental requirements in the first year of life. Infants progress from an exclusively liquid diet to a mixed varied diet in a relatively short space of time.

In the UK it is recommended that this process commences around six months of age, but not before 17 weeks of age (Department of Health, 2003). The timing of introduction of solid food may be influenced by a number of maternal and infant factors; such as health professional advice, advice from friends and family, socioeconomic status, knowledge of guidelines, perception of infant needs, personal and cultural beliefs (Arden, 2010; Moore, Milligan, & Goff, 2014). It is thought that those who are introduced to solid foods earlier may develop different dietary patterns as they are "fast tracked" through the process, resulting in consumption of adult foods at an early age (Howard, Mallan, Byrne, Magarey, & Daniels, 2012; Robinson et al., 2007). Other weaning factors such as repeated exposure, variety, texture and method of weaning will now be discussed in relation to development of food preferences and eating behaviours.

3.9.1 The role of repeated exposure and variety during complementary feeding

During the complementary feeding period, an infant will usually be exposed to a wide range of novel tastes and textures. At the beginning of the weaning process all foods are new. Throughout the complementary feeding process, it can be said that infants "learn to like" flavours as innate preferences and aversions interact with food exposures. Although breastfed infants have an advantage to initially accept solid food if already exposed to the taste, both formula and breast fed foods respond well to repeated exposure to a food (Mennella & Trabulsi, 2012). Indeed, it is thought that repeated exposure to a food is one of the primary determinants of its acceptance (Nicklaus, 2011). Repeatedly presenting a food provides an opportunity to eat the food whilst associating it with a positive social context (Birch, 1999). In contrast, negative learned associations can occur if there are negative post-ingestive effects (e.g. vomiting) from consuming particular foods (Birch, 1999; Wardle & Cooke, 2008). This is very relevant in food allergy, particularly if there is a delay to diagnosis meaning negative experiences occur repeatedly.

Two highly cited studies illustrate how exposure can lead to increased acceptance of foods and the differences in number of exposures required at different ages (Birch, Gunder, Grimm-Thomas, & Laing, 1998; Birch & Marlin, 1982). In the first study, infants aged 4-7 months old (n = 39) were fed a target food (either banana or peas) once a day for ten days (Birch et al., 1998). Exposure dramatically increased infants' intake of the target food, doubling the portion

eaten. The same study also looked at the effect the exposure to a target food had on the acceptance of a similar (a different fruit or a different vegetable) or a different food (fruit, vegetable or meat, depending on the target food used). The results demonstrated an increase in consumption for a similar, but not for a different food. These findings imply that during the early stages of complementary feeding preference for a food via exposure occurs very quickly and liking can extend to foods within the same category.

A second landmark study by the same research group demonstrated that as infants progress into their second and third year of life, the number of exposures required to produce preference for a food increases. A study of two year old children using five different types of novel cheese found that significant increases in preference occurred after 5-10 exposures (Birch & Marlin, 1982). Overall the 'exposure effect' is described as consistent, powerful and universal (Nicklaus, 2011). However it is known that in reality most foods are only presented approximately five times before a parent decides the child dislikes the food (Carruth, Ziegler, Gordon, & Barr, 2004), meaning in many cases the exposure is not prolonged enough to promote preference.

In addition to repeated exposure to foods, the variety of foods offered is also known to have an influence on acceptability. Early experience with a diversity of flavours may lead to an increased readiness to accept unfamiliar foods, which is likely to increase the range of nutrients consumed (Gerrish & Mennella, 2001). In a study of formula-fed infants, those who were randomised to be fed a variety of vegetables that differed in taste, smell and texture ingested more of a novel food (chicken) than those who were only fed one type of vegetable. This provides evidence for the common advice health professionals give to parents to offer a variety of flavours and colours in the early stages of weaning.

Even though these studies used healthy participants, who had no dietary restrictions, there remains obvious implications for infants and children with food allergies who cannot be exposed to certain tastes and flavours and therefore may have reduced dietary variety. A challenge of familiarising children with new foods and tastes is that sampling the food is necessary to alter preference, yet children are often reluctant to try new foods (Birch & Doub, 2014) . This has particular relevance in previously food allergic children who may have been told repeatedly by parents not to eat a specific food as it will make them ill.

3.9.2 The role of texture in complementary feeding

Texture, in addition to appearance, taste and odour, plays a role in the acceptance (and rejection) of foods. Although some texture assessment is performed visually, the main

evaluation occurs in the mouth (Szczesniak, 2002). Babies and young children reject textures that are difficult to manipulate in the mouth at a particular stage of physical development. Stringy, gummy or slimy foods or those containing unexpected lumps or hard particles are rejected for fear of gagging or choking (Szczesniak, 2002). Additionally children may dislike food with bits or pips which might indicate contamination (Wardle & Cooke, 2008). Exposure to different textures may be limited because of maternal anxiety of choking (Cameron, Heath, & Taylor, 2012). This anxiety may be heightened in infants who have a history of reflux and vomiting.

Generally complementary feeding progresses through a number of distinct stages of different textured foods. Advice in the UK suggests weaning should begin with smooth foods at around six months, before progressing to mashed foods with lumps (7-9 months) and eventually transition to chopped foods (around 9-12 months) (Department of Health, 2003). In recent years, there has been a trend towards an alternative method of weaning; “baby led weaning” (BLW). This is the process through which babies feed themselves small finger sized pieces of food and choose the pace of solid food introduction from the outset rather than being spoon fed with pureed food (Brown & Lee, 2011). Despite the anecdotal popularity of BLW, nationally representative data indicates that in 2010, only 4% of infants in the UK were given finger foods as their first food, with 94% fed mashed or pureed foods, with baby rice being the most common food introduced first (Mc Andrew et al., 2012). However it may be that the two approaches to weaning are not mutually exclusive and that in reality a combination of both approaches is used, with a continuum where spoon-feeding is used predominantly to not at all.

It is thought that if infants are not introduced to chewable foods at the recommended age they may be less likely to accept new textures at a later age (Northstone, Emmett, & Nethersole, 2001). In a study of the Avon Longitudinal Study of Parents And Children (ALSPAC) cohort (n = 9360), 10.7% of infants were introduced to lumpy food before six months of age and 71% introduced between six and nine months. Those who delayed introduction to lumpy foods to after nine months were significantly less likely to be having family foods by 15 months of age, had definite food dislikes and were more difficult to feed. They were also more likely to be given sweetened foods such as infant and adult puddings regularly (Northstone et al., 2001). However, it was also shown in this study that those introduced early to lumps were fed unsuitable foods such as crisps, chocolate and tea before six months of age and salty foods such as processed soup and gravy at 15 months, suggesting both early (before six months) and late (after nine months) introduction of textured food may have negative associations with later food consumption.

A subsequent study from the same cohort demonstrated that the delayed introduction of lumpy foods may have a persistent effect on dietary intake and eating behaviour (Coulthard, Harris, & Emmett, 2009). Those who were introduced to lumpy foods after nine months of age had a lower consumption of fruit and vegetables at age seven years and consumed fewer different types of vegetables (Coulthard et al., 2009). There was no detrimental effect of introducing lumpy foods before six months at age seven years. A more recent publication analysing patterns of dietary intake from the same cohort found that children who were introduced late to lumpy foods scored lower on "family food" (meat, fish, puddings, potato and vegetables) and "health conscious" (fruit, vegetables, eggs, nuts and juices) patterns of dietary intake at two years old (Northstone & Emmett, 2013). However scores for each dietary pattern were clearly associated with socio-demographic variables (e.g. maternal education). It also should be highlighted that these studies took place with infants born in 1990/1991 and since then weaning guidelines in the UK have changed (Department of Health, 2003). Nevertheless it provides important evidence that a sensitive window of opportunity may exist with regards to food texture, in a well-established cohort study using a UK population.

3.9.3 Mode of weaning: traditional or baby led

Although this thesis does not specifically address or measure BLW, it is a practice that warrants some discussion in the context of infant feeding behaviour in general. Despite the interest in BLW, a review of the literature indicated there is a paucity of studies directly investigating its effect on nutritional intake and feeding behaviour and many questions remain (Cameron, Heath, & Taylor, 2012). Proponents of this method suggest that BLW may encourage improved eating patterns by encouraging greater acceptance of varied textures and flavours (Cameron et al., 2012). However this theory is confounded by the fact that mothers who use a BLW approach are more likely to breastfeed, be more educated and return to work later (Brown & Lee, 2011). Concerns raised against BLW are the risk of choking, and inadequate energy and iron intake (Cameron et al., 2012). A recent pilot study of a modified version of BLW suggests a greater number of iron containing foods were offered and a lower choking risk incurred compared to a traditional BLW approach, however numbers in the study were very small (n = 23) and study groups were self-selected (Cameron, Taylor, & Heath, 2015).

One study was identified which investigated the effect of weaning style on food preferences and BMI using a case controlled study design of 155 children between 20-78 months old (Townsend & Pitchford, 2012). The authors reported that the BLW group had significantly increased liking for carbohydrates, attributed to the use of toast/pitta bread as an

early finger food. Those in the spoon-fed group favoured sweet foods the most, which could be explained by the fact that the majority of commercial baby food available in the UK are sweet (García, Raza, Parrett, & Wright, 2013). There was no difference in fussy eating between the two groups. Interestingly there was a higher prevalence of obesity in the spoon fed group and correspondingly a higher prevalence of underweight in the BLW group. The study findings are limited by the fact that the infant feeding data was collected retrospectively using a self-report method and classified arbitrarily into spoon-fed or BLW. Additionally there was no discrimination made between spoon feeding homemade or commercially produced baby food. However despite the limitations, the findings emphasise the importance of early exposure and familiarity in predicting food preferences.

3.9.4 Parental influence during complementary feeding

Parents influence their offspring's eating habits in two broad ways: through genetics (heritability of traits) and through the environment factors (feeding practices used).The heritability of different eating traits will be discussed in the later sections of this chapter on fussy eating, feeding difficulties and food neophobia. The influence of the parental and family environment on feeding behaviour will now be briefly discussed.

What, when and how parents feed their child plays a critical role in the formation of food preferences and eating behaviours (Birch & Doub, 2014). As an infant develops into a child and an adolescent, more autonomy over food choice is gained. However in infancy dietary intake is limited by what is offered and available. Children learn a considerable amount of information about food and eating during the first few years of life, from likes and dislikes, to portion sizes, to timing of meals, to what foods are consumed at what mealtimes and what foods are eaten in combination (Birch, 1999). Much of this is learnt through exposure and imitation. Evidence highlighting the importance of parental food intake, feeding style and availability of different foods was demonstrated by a twin study, overall concluding that shared environmental factors explained 82-95% of the variation in consumption of different food groups in toddlers (Pimpin et al., 2013).

Parents' food preferences and eating behaviours provide an opportunity to model good eating habits. Conversely, practices used by parents such as pressure to eat certain foods, restriction by limiting access to certain foods and use of food as a reward are associated with negative outcomes (Scaglioni et al., 2011). Parents are seen as "gatekeepers" of their child's food environment (Webber, Hill, Saxton, Van Jaarsveld, & Wardle, 2009). Patterns of infant feeding are associated with parent and family characteristics, most notably the maternal diet

(Robinson et al., 2007). In a large study of women and children in the South of England, two main dietary patterns were identified at six and twelve months of age: “infant feeding guidelines” (characterised by vegetables, fruit, meat, fish, home prepared foods and breast milk) and “adult foods” (characterised by bread, savoury snacks, biscuits, squash, breakfast cereals and chips). At twelve months and three years of age, the quality of the maternal diet was the most influential factor on the child’s diet, explaining a third of the variance in the child’s dietary quality (Fisk et al., 2011). Although this study measured food intake rather than preference, concordance in maternal and infant food preferences has been reported in other studies and it is hypothesised that mothers’ own food likes strongly influence whether they offer their child a particular food or not (Howard et al., 2012).

The majority of studies of child eating behaviour have only assessed maternal factors or used maternal factors as a proxy for both parents, which could be a source of bias (Khandpur, Blaine, Fisher, & Davison, 2014). On the whole, in studies that directly compared mothers and fathers feeding practices, it was concluded that fathers focused on getting children to eat and were less concerned about the types of foods consumed than mothers (Khandpur et al., 2014). In practical terms, dependent on a mother’s work commitments and family routine, a large proportion of a young child’s mealtimes may be supervised and therefore influenced by other adults (e.g. childminders or grandparents). A study exploring non-maternal caregivers reported a decreased likelihood of continued breastfeeding and an increased likelihood of infants and toddlers consuming juice or whole fruit (Wasser et al., 2013), thus demonstrating a wide spectrum of influences on infant eating habits.

3.9.4.1 Parental feeding practices

Recent qualitative research suggests parents use many diverse behaviours to influence their child’s food preferences, some of which may be effective (e.g. parental modeling, encouragement or food exposure) (Russell, Worsley, & Campbell, 2015). Conversely, parents can control feeding practices in a number of negative ways i.e. use of preferred foods as a reward, restriction of access to unhealthy foods and/or pressure to consume healthy foods (Collins, Duncanson, & Burrows, 2014). The use of controlling feeding practices is thought to desensitise children to their internal cues of satiety, decreasing their ability to regulate their intake of food. Parents who manage their child’s limited diet by offering their preferred food only further reinforce the child’s avoidance of unfamiliar foods (Scaglioni et al., 2011). Indeed the practice of separating children’s and adults food consumption by offering children only the foods they liked, allowing children to eat different meals than adults or offering children alternatives

to rejected foods are behaviours associated with unhealthy food consumption in preschool children (Russell et al., 2015)**.**

Within families, it has been suggested that parents use different levels of pressure and restriction with different children depending on their behavior, temperament and weight. Greater levels of restrictive feeding practices and more pressure to eat are used in fussier children in comparison to their sibling, which is likely to have a counter productive effect by exacerbating the fussiness (Farrow, Galloway, & Fraser, 2009). However it is likely the relationship is bidirectional and cyclical, whereby fussy and avoidant eating behavior results in parental prompts to eat more, further reducing the child's willingness to eat (Powell, Farrow, & Meyer, 2011). A study of 7-9 year old children in London indicated that maternal pressure to eat was associated with lower enjoyment of food, slowness in eating and fussiness, after controlling for child BMI (Webber et al., 2009). However, these studies are all cross-sectional and the relationships are associative rather than predictive. A systematic review on this topic only identified seven studies that met their criteria, overall concluding that parental styles showed weak to moderate associations with individual aspects of child feeding behavior (Collins et al., 2014).

3.10 Fussy eating

3.10.1 Definition of fussy eating

Fussy eating is defined as the "consumption of an inadequate variety of foods through rejection of both familiar and unfamiliar foods" (Dovey et al, 2008). It may also be described as 'picky', 'faddy' or 'selective' eating and the terms are used interchangeably. Several variations of the definition exist, with no exact consensus, but it is typically characterised by strong food preferences, reduced dietary variety and dislike of certain food groups, usually vegetables (Taylor, Wernimont, Northstone, & Emmett, 2015). Features may also include longer mealtimes and concerns regarding food presentation and preparation (Jacobi, Schmitz, & Stewart Agras, 2008). Some definitions include a temporal criteria e.g. Chatoor et al. (2003) defines picky eating as food refusal "for at least one month" and Bryant Waugh (1999) defines selective eating as "eating a narrow range of foods for at least two years".

In most young healthy children fussy eating can be viewed as a mild or transient problem that is not considered a medical condition (de Moor, Didden, & Korzilius, 2007; Kerzner et al., 2015). Similarly, in older children aged 8-12 years old, picky eating is not related to disturbed eating practices such as dieting or binge eating and is not a precursor to adolescent

eating disorders (Jacobi et al., 2008). Although it may be dismissed as a benign issue by health professionals and researchers, fussy eating is significantly associated with caregiver stress and is perceived to cause family conflict and have a negative impact on family relationships (Goh & Jacob, 2012; Zucker, Copeland, Franz, Carpenter, & Keeling, 2015). Parents of picky eaters are often concerned about both the physical and mental consequences of fussy eating on their child's health, prompting some of them to seek health professional advice (Goh & Jacob, 2012; Wright, Parkinson, & Drewett, 2006).

3.10.2 Prevalence of fussy eating

Food refusal is commonly seen during infancy. As is the case with prevalence estimates for any behaviour or condition, the figures vary widely dependent on the age, the study population, the country and method of assessment used. A recent review found that only two prevalence studies have taken place in a UK population (Taylor et al., 2015).

The first study, of children mean age 30 months, from the millennium birth study in Newcastle reported that a fifth of parents perceived their children as having eating problems. The most commonly cited problems were "eats a limited variety of food" and "prefers drinks to food" (Wright, Parkinson, Shipton, & Drewett, 2007). Thirteen per cent of parents had sought professional help for this. Overall 8% of the parents in the study described their child as being "definitely faddy". Those who were described as "faddy" liked on average 15 foods less than those not described as faddy. Faddy eating was apparent across the whole cohort, with no difference in prevalence according to socioeconomic or educational status. However the response rate for the study was only 49%, indicating the possibility of a response bias.

The second UK study is from the ALSPAC group, where the prevalence of fussy eating varied with age from 10% to 15%, with the peak prevalence at 38 months (Taylor et al., 2015). Unlike the millennium cohort study, associations were found for sociodemographic variables. Picky eating was associated with greater maternal age, maternal smoking, higher maternal social class, lower pre-pregnancy body mass index, higher maternal educational attainment, lower parity, and the infant being male and of a lighter birth weight. Different findings between the two studies may be due regional differences, study design and methodology.

Studies from other countries have shown that parent-perceived fussy eating is as high as 50% at ages 19-24 months in the USA (n = 3054) (Carruth et al, 2004). Zucker et al. (2015) recently reported that 17% and 3% of pre school children are classed as "moderate" and "severe" selective eaters respectively, with rates ranging from 14-17% in children between the ages of two and four years in Canada (n = 2103) (Dubois, Farmer, Girard, Peterson, & Tatone-

Tokuda, 2007). In a study of 1-10 year old children from Singapore, half of the respondents reported their child was 'all the time' (25.1%) or ‘sometimes’ (24.1%) a picky eater (Goh & Jacob, 2012). Respondents who had professional occupations were significantly more likely to perceive their child to be a picky eater, compared to respondents from other occupations.

In the Netherlands, a prevalence of 5.6% was reported in four year old children, with higher rates of fussy eating in boys, those from a lower socioeconomic background and with younger mothers (Tharner et al., 2014). A different Dutch cross sectional study of toddlers (n = 422) reported that 65% had at least one feeding problem; 58% of the feeding problems were mild, whereas 7% were moderate to severe (de Moor et al., 2007). No statistically significant associations were found between picky eating and gender, types of childcare arrangements or ethnic background of either parent. The strikingly different prevalence rates of 5.6 and 65% reported in two different Dutch studies, highlights the problem of inconsistency in methodologies used. Tharner et al. (2014) used a validated questionnaire, the Child Eating Behaviour Questionnaire (which will be used in this thesis), whereas de Moor et al. (2007) classified children as fussy if they answered positively to only one questionnaire item. Differences in methodology will be discussed in more detail in chapters four and five.

As fussy eating is typically thought to be a problem in early childhood, there are fewer studies of older children. A German study of 8-12 year old children reported that 19% of girls and 18% of boys were picky eaters, again using a maternal report methodology (Jacobi et al., 2008). A Chinese study of 794 children aged 7-12 years old reported a very high prevalence of 59%, however classification as a fussy eater was based on a simple yes/no question (Xue et al., 2015).

Most of the aforementioned studies of fussy eating are cross-sectional, however in the few longitudinal studies, parents have reported an increased perception of their child’s pickiness as they get older: from 25% at 7-8 months to 35% at 12-14 months to 50% at 19-24 months (Carruth et al., 2004), which may be because the child has the ability to verbalise his/her dislikes as they get older (Dovey et al., 2008). Cardona Cano et al (2015) reported a peak in fussy eating prevalence at age three years, finding rates of 26.5% at one year, 27.6% at three years and 13.2 % at six years. Amongst children categorised as fussy eaters, 55% were picky at all three stages, with boys and those from ethnic minorities more likely than girls to be persistent picky eaters. Mascola, et al. (2010) studied 120 children from age 2-11 years, finding a peak of fussy eating prevalence at age six years, with 40% of children being described as a fussy eater for a duration of two years, indicating the chronicity of the problem. The tracking of fussy eating will be discussed further in chapters four and five.

3.10.3 Measurement of fussy eating

As can be seen, differences in methods used leads to a wide range in reported prevalence. Fussy eating can be difficult to quantify accurately. Some studies use dichotomous measures, whilst others use continuous scales. Galloway et al. (2003) used a combination of a continuous and a dichotomous measure, by arbitrarily splitting the data into two categories of “picky” and “non picky” at the median score of a questionnaire scale. Multi item scales provide more detailed data regarding specific eating behaviours, but the lack of a validated cut off to classify “fussy” and “non fussy” can limit the interpretability of results and clinical implications (Tharner et al., 2014). Proponents of studies that use binary questions (yes/no) argue that it does not rely on a parental/caregiver interpretation of the term ‘picky eating’, or on parental/caregiver interpretation of time (e.g. slow eating) (Taylor et al., 2015).

An additional limitation of all of these studies is that parental self-report methods are used rather than direct observation of eating. Tools such as the Children’s Eating Behaviour Questionnaire (CEBQ) (Wardle, Guthrie, Sanderson, & Rapoport, 2001) (see appendix 9) and Carruth’s Picky Eater questionnaire (Carruth et al., 1998) (see appendix 10) have been validated against behavioural measures of eating at different age ranges (Blossfeld, Collins, Kiely, & Delahunty, 2007; Carnell & Wardle, 2007). Other questionnaires used in published studies have not necessarily been validated (Goh & Jacob, 2012; Wright et al., 2007; Xue et al., 2015). This is problematic as many young children are perceived by parents to eat poorly (Kerzner et al., 2015), which may be because parental expectation of food consumption is unrealistic, rather than being a true reflection of inadequate dietary intake. A more detailed description of the various measurement tools used for assessing fussy eating status is found in chapter four.

3.10.4 Effect of fussy eating on dietary intake

Studies investigating the effects of fussy eating have reported their data in different ways. Some have focused on food groups, some have evaluated nutritional intake, and some have reported both. The data derived from nutritional assessment is highly dependent on the method used, which will be discussed in more detail in chapters four and five.

3.10.4.1 Effect of fussy eating on food group consumption

Despite the fact that studies have taken place in different countries and different age groups, the data regarding avoidance of food groups and fussy eating is very consistent. All studies report relatively low levels of intake of vegetables (Cardona Cano et al., 2015; Carruth et al., 1998; de Moor et al., 2007; Galloway, Fiorito, Lee, & Birch, 2005; Jacobi, Agras, Bryson, &

Hammer, 2003; Tharner et al., 2014) and many also report lower intakes of meat and fish, potatoes and rice/pasta (Cardona Cano et al., 2015; de Moor et al., 2007; Tharner et al., 2015). There is also a lower reported intake of composite dishes amongst fussy eaters (Carruth et al., 1998).

None of the studies of fussy eating in younger children report a difference in consumption of fruit or dairy products. In contrast, in older children, picky eaters were found to avoid more dairy products, fruits, vegetables, meat, fish, fast food noodles, potatoes, rice and beverages i.e. pickiness in 8-12 year olds was related to *all* foods in general, rather than specific categories of food (Jacobi et al., 2008). In terms of what food groups fussy eaters *do* eat, it has been reported that they tend to consume more sweetened foods and confectionary (Cardona Cano et al., 2015; Carruth et al., 1998) and savoury snacks (Tharner et al., 2014). This has been attributed to mothers of fussy eaters being more permissive in allowing child to eat palatable but unhealthy foods to compensate for lower intake or other foods (Tharner et al., 2014).

3.10.4.2 Effect of fussy eating on nutritional intake

Nutrient intake of fussy eaters appears fairly similar to non fussy eaters in most aspects (Dovey et al., 2008). Indeed one author has concluded that the major concern with fussy eaters is not nutrition, but coercive feeding that parents employ and the subsequent behavioural consequences (Kerzner et al., 2015). Differences in research design mean that some studies have used a control group, but others have used national dietary recommended intakes as a comparison. No studies were identified in a UK population.

In infants, it was shown that both picky and non picky eaters between 7-11 months met or exceeded energy requirements, but subgroup analysis of 9-11 month olds indicated a lower intake of energy, fats and micronutrients in the picky eater group (Jacobi et al., 2003); suggesting that at a younger age milk or formula may make more of a contribution to nutritional requirements. In toddlers, Carruth et al. (1998) found that intakes of calcium, zinc, vitamin D and vitamin E were below US recommended intakes in both picky and non-picky eaters, but there were no significant differences between the two groups. In a large study of Canadian pre school children, who were assessed at two, three and four years old, picky eaters consumed fewer fats, less energy and less protein than non-picky eaters (Dubois et al., 2007), but the authors did not explain how this related to nutritional recommendations thus making it difficult to interpret the results in context.

Looking at older studies of older children, Galloway et al. (2003) found no differences

in energy or macronutrient intake in picky and non-picky eater nine year old girls. Intakes of vitamin E, calcium and magnesium were below recommended intakes in both groups, but intakes of vitamin E and folate were significantly lower in the picky group than the non-picky group. Whether the same trends would be observed in boys is unknown. A Chinese study of 7-12 year old children reported that picky eaters had a lower dietary intake of energy, protein, carbohydrates, most vitamins and minerals, and lower levels of magnesium, iron, and copper in the blood, compared to non picky eaters (Xue et al., 2015). This was despite the fact that nutritional supplementation was higher in the picky eating group. However dietary intake was based on only one 24-hour recall and intakes were not compared to nationally recommended requirements. Interestingly, in their recent review of 60 studies about fussy eating, Taylor et al. (2015) note several nutrients have not been reported, such as selenium, iodine, sodium and sugars; which could be due to a paucity of studies or a lack of difference between groups.

3.10.5 Fussy eating and growth

Mothers of picky eaters are said to be worried about their child's weight being low (Goh & Jacob, 2012; Jacobi et al., 2008). Surprisingly, one definition of fussy eating states "no growth deficiency" as one of the criteria (Chatoor, 1998) whereas another definition is more ambiguous, stating that weight can be "low, normal or high" in fussy eaters (Bryant-Waugh, 1999). Overall mixed findings have been reported in the literature, again likely to be due to the heterogeneity of methods used to quantify fussy eating.

Two studies of 2-3 year old children illustrate this heterogeneity very well. Firstly in the UK millennium birth cohort study, children who were described as having an "eating problem" gained less weight over the first two years; 11% had weight faltering, compared to 3.5% in children not described as having an eating problem (Wright et al., 2007). However in those specifically described as "faddy eaters", no significant difference in growth was reported, thereby illustrating the issue with not clearly differentiating between "problem eating" and "faddy eating". Secondly, an Israeli study of preschool children referred to a clinic for fussy eating reported 20% of the picky eating group were underweight compared to 6% of the control group (Ekstein, Laniado, & Glick, 2010). However the study design, with participants recruited from a feeding clinic rather than an unselected population means the picky eating group are likely to be skewed towards the more severe end of the spectrum, as not all parents seek health professional advice and attend a clinic.

Looking at population based studies, a Dutch study of four year old children found those in the fussy eating category were more likely to be underweight (19% vs. 12%) (Tharner et al.,

2014). Similarly, a study of four year old children in Canada reported picky eaters were twice as likely to be underweight than non picky eaters (Dubois et al., 2007). A different Dutch study of five year old children (n = 621) reported that picky eaters were more like to be shorter and underweight than non picky eaters and less likely to become overweight by age nine (Antoniou et al., 2015). In older children, BMI did not differ by picky eating status in children aged 8-12 years old in Germany (Jacobi et al., 2008). In a large Chinese study of school-aged children (n = 814), the authors extrapolated that picky eating behavior was associated with a reduction of 1.29 cm in height and 2.85 kg in weight (Xue et al., 2015). However the methods used to classify children as picky or non-picky eaters in this study was very arbitrary, using only a single parent-report question as the criteria.

Although typically researchers and clinicians associate fussy eating with underweight, it may be that fussy eating is associated with overweight and obesity if low calorie snacks (e.g. fruit) are displaced with higher fat choices. One study of 2-6 year old children found higher rates of fussy eating in overweight and obese children than normal weight children (Finistrella et al., 2012), whereas a study of seven year old girls found picky eaters were less likely to be overweight (Galloway et al., 2005). However only one participant in the whole sample was underweight, therefore it can be said that results need to be interpreted in the context of the norms of the population studied. In a UK study of 7-12 year old children that was primarily investigating overweight and obesity, food fussiness was negatively associated with weight; reporting that fussy eating may be “protective” against obesity (Webber et al., 2009). However the authors noted that the participants in the study were relatively lean compared to the UK average, suggesting that a self-selection bias may exist with overweight and obese children not taking part.

3.10.6 Fussy eating and food allergy

All of the aforementioned studies have involved healthy participants. Yet it is known that children with medical conditions such as autism spectrum disorder or cystic fibrosis may display fussy eating traits (Bandini et al., 2010; Powers et al., 2005). Very few published studies have addressed the issue of fussy eating in food allergic children (Haas, 2010). Indeed only one study was identified.

A large study of school age children in China reported data on food allergic children as a subgroup (Xue et al., 2015). A significant difference in food allergy history was found between the two groups, with 9.2% of those with a food allergy history being a picky eater compared to 6.5% of those without a food allergy history. However the information about food allergy was

collected via parental self-report, not from clinical examination, as food allergy was not the focus of the research. The authors did not clarify whether the food allergy was current or outgrown and although they documented which foods were implicated, there was no analysis performed of differences between foods or numbers of foods excluded (personal communication Yumei Zhang August 2015). The distinct lack of studies in this topic underlines that it is an area that warrants further research.

3.11 Feeding difficulties

3.11.1 Definition and classification of feeding difficulties

In healthy infants and toddlers, it is known that development of feeding skills occurs between 0-24 months. There is individual variation in gaining self-feeding fine motor skills, meaning infants progress at different rates and some will display challenging behaviour during this process (Carruth & Skinner, 2002). “Feeding difficulties” refers to a spectrum of problematic eating behaviours such as excessive spitting out of food, crying/irritability at feeding time, eating extremely slowly, retching at the sight of bottle or spoon, apparent difficulty in swallowing, throwing and pushing away food (Crist & Napier-Phillips, 2001; Lewinsohn et al., 2005). Feeding difficulties arise because of a complex interaction of biological, social and behavioural issues (Crist & Napier-Phillips, 2001). Fussy eating and feeding difficulties are separate entities, but they may co exist. Feeding difficulties do not necessarily delay the child’s development, but may be the start of poor eating habits in which mother-child interaction plays a key role (Esparó et al., 2004) Features of feeding difficulties and indications of more serious “red flag” presentations are shown in Table 3.2 (Kerzner et al., 2015).

Table 3.2 Presenting features of feeding difficulties versus "red flag" eating behaviours

Suggestive Symptoms/Signs	**Organic red flags**	**Behavioural red flags**
Prolonged mealtimes	Dysphagia	Food fixation (selective, extreme dietary limitations)
Food refusal lasting < 1 month	Aspiration	Noxious (forceful and/or persecutory) feeding
Disruptive and stressful mealtimes	Apparent pain with feeding	Abrupt cessation of feeding after a trigger event
Lack of appropriate independent feeding	Vomiting and diarrhea	Anticipatory gagging
Nocturnal eating in toddler	Developmental delay	Failure to thrive
Distraction to increase intake	Chronic cardio-respiratory symptoms	
Prolonged breast or bottle feeding	Growth failure	
Failure to advance textures		

3.11.1.1 Differentiation between feeding difficulties and feeding disorders

Similar to fussy eating, the measurement of feeding difficulties can be inconsistent due to the variability in definitions used. In many cases feeding difficulties are transient; however it is not always easy to differentiate feeding problems that are likely to be short-lived from those that are more persistent (Bryant-Waugh, Markham, Kreipe, & Walsh, 2010). By comparison, the term ‘‘infant feeding disorder’’ is a formal diagnosis used in the current diagnostic system of the World Health Organisation International Classification of Diseases-10 (World Health Organisation, 2015). It is described as a persistent failure to eat adequately, involving extreme faddiness, that is not directly due to a medical condition or another mental disorder or due to lack of availability of food, with onset before six years of age.

A similar definition was also previously published in the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM IV) (American Psychiatric Association, 2000). It stated that the diagnosis of “feeding disorder of infancy or early childhood” should be made only if the eating problem results in significant failure to gain weight or loss of weight. In 2013, the diagnosis was updated in the new edition of the book (DSM-V) and the terminology of infant feeding disorder was changed to “Avoidant/Restrictive Food Intake Disorder” (AFRID) (American Psychiatric Association, 2013). AFRID is a broad category intended to capture a range of presentations that have restrictive intake with malnutrition but no body image

disturbance or fear of weight gain. The diagnosis of AFRID is applicable across the lifespan and not limited to infancy. The criteria for AFRID is shown in Table 3.3 (American Psychiatric Association, 2013).

As many children who consume exclusion diets maintain a normal weight and have an underlying disorder (i.e. food allergy), the use of these definitions for “infant feeding disorder” and AFRID (WHO ICD 10 and DSM V) were not appropriate for this study. Other classification systems for feeding disorders are the Chatoor criteria, which has six different subdivisions and the Wolfson criteria, which is slightly simpler (Levine et al., 2011).

Table 3.3 Diagnostic criteria for Avoidant/Restrictive Food Intake Disorder

Criteria
-An eating or feeding disturbance (e.g., apparent lack of interest in eating or food; avoidance based on the sensory characteristics of food; concern about aversive consequences of eating) as manifested by persistent failure to meet appropriate nutritional and/or energy needs associated with one (or more) of the following: -Significant weight loss (or failure to achieve expected weight gain in children). -Significant nutritional deficiency. -Dependence on enteral feeding or oral nutritional supplements. -Marked interference with psychosocial functioning. -The disturbance is not better explained by lack of available food or by associated culturally sanctioned practice. -The eating disturbance does not occur exclusively during the course of anorexia nervosa or bulimia nervosa, and there is no evidence of a disturbance in the way in which one’s body weight or shape is experienced. -The eating disturbance is not attributable to a concurrent medical condition or not better explained by another mental disorder. When the eating disturbance occurs in the context of another condition or disorder, the severity of the eating disturbance exceeds that routinely associated with the condition or disorder and warrants additional clinical attention.

3.11.2 Measurement of feeding difficulties

Assessment of feeding difficulties traditionally involves anthropometry, direct observation of mealtimes and parent questionnaires regarding dietary behaviours (Marshall, Raatz, Ward, & Dodrill, 2014). A comprehensive description and discussion of different assessment tools will be included in chapter four, but to summarise; there is a lack of consistency in screening tools and many are not validated against behavioural measures.

3.11.3 Prevalence of feeding difficulties in healthy children

As is the case with fussy eating, the parental perception of feeding difficulties is higher than the actual prevalence of feeding difficulties. Differences in criteria and nomenclature make estimations of prevalence unreliable. In the UK population, 40% of parents from the ALSPAC cohort reported "feeding problems" in their children at 15 months, however a non-validated method was used (Northstone & Emmett, 2013). Similarly Wright et al. (2007) reported that 59% of one year old children push food away, 54% spit food out and 41% hold food in the mouth, however the authors acknowledge that the method used was crude and non-standardised.

Studies from other countries have cited that difficult mealtime behaviours occur commonly in normally developing children, with 21% of parents reporting four or more behaviours (Crist & Napier Phillips, 2001), the most common being "gets up from table during meal", "eats junky snack foods but will not eat at mealtime", "whines or cries at feeding time". In a small study (n = 93) of healthy three year old children, although all children were able to feed themselves with a spoon and fork and able to drink from a cup, 78% of parents reported that their child spits out food, 49.5% of children throw tantrums when they are refused food, 37.6% choke on food whilst eating and 35% push away or throw food (Lewinsohn et al., 2005).

In a more recent study of 402 healthy children aged 1-4 years old in Thailand (Benjasuwantep, Chaithirayanon, & Eiamudomkan, 2013), feeding difficulties were reported in 26.9% of children. Those who had feeding difficulties were found to be fed less frequently, to be less likely to be fed at a table and to have mealtimes longer than thirty minutes. The prevalence was highest in the second year of life, after which it gradually decreased. This was attributed to the fact that after the second year the child gains increased experience manipulating and accepting food and are less easily distracted. In contrast, using the strict DSM-IV criteria (American Psychiatric Association, 2000), in a sample of 1104 preschool children in Spain, it was shown that the prevalence of infant feeding *disorders* was 4.8% (Esparó et al., 2004). This discrepancy is illustrated in Figure 3.4, taken from Kerzner et al.

(2015), where it can be seen that approximately 25% of children will be identified to have feeding difficulties by parents, but only 1-5% at the apex will meet criteria for a feeding disorder.

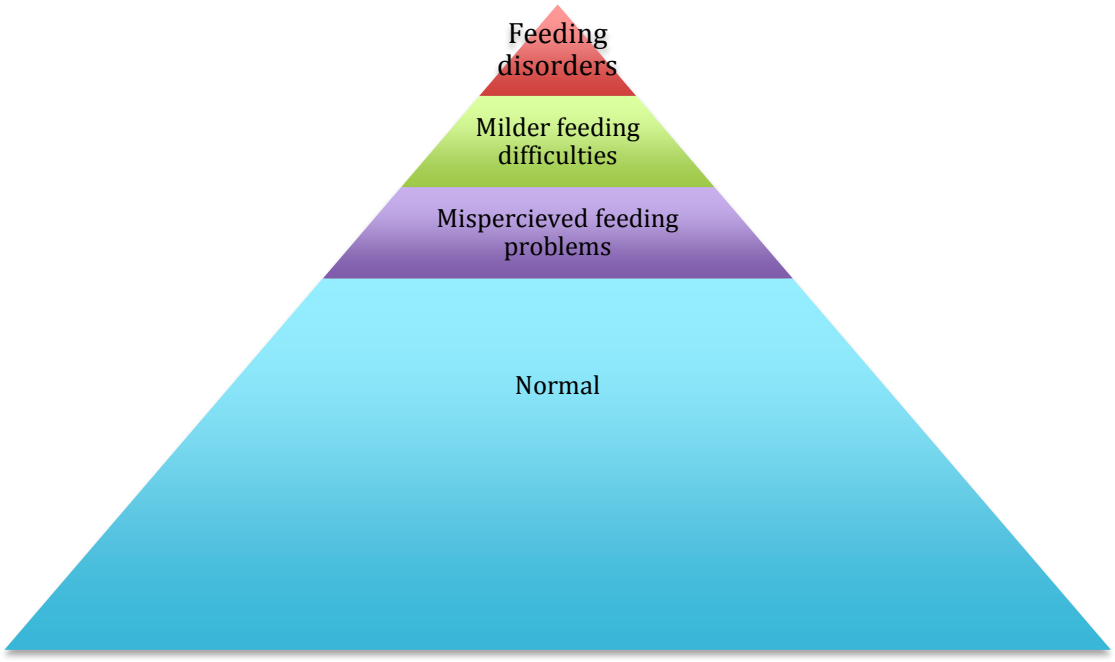

Figure 3.4 Pyramidal representation of young children's feeding behaviours

3.11.4 Feeding difficulties, nutritional intake and growth

Much of the literature regarding feeding difficulties is concerned with the fundamentals of defining it and ensuring a consistent classification for diagnosis, rather than assessing the nutritional consequences. No studies were identified that reported the specific nutritional intake of children with feeding difficulties, except to state that reduced dietary variety is a defining feature or that artificial feeding is required in severe cases (Rommel, De Meyer, Feenstra, & Veereman-Wauters, 2003).

In terms of growth, in a UK birth cohort study exploring faltering growth (n = 923), there was an association between avoidant eating behaviour and weight gain at age twelve months (Wright et al., 2006). However in a multivariable model the only significant predictor of sustained weight faltering at twelve months was the maternal response to food refusal, implying that high maternal promotion of feeding may have an adverse effect. Therefore it may be the *response* to the feeding difficulties rather than the feeding difficulties itself that cause poor growth. The discrepancy of research in this are compared to the perceived prevalence of the problem, emphasises the need to investigate this topic further.

3.11.5 Feeding difficulties in children with medical conditions

Although feeding difficulty behaviours occur routinely in healthy children, there is a high prevalence of comorbidity. Feeding difficulties can persist even once the presenting medical condition is treated (Levy et al, 2009). Feeding difficulties have been documented in children with a range of different medical conditions, including, but not limited to: cystic fibrosis (Powers et al., 2005), autism spectrum disorder (Emond, Emmett, Steer, & Golding, 2010), inherited metabolic disorders (Evans et al., 2012) , type 1 diabetes (Powers et al., 2002) and ex premature infants (Migraine et al., 2013). Additionally healthy children born small for gestational age were more likely to have feeding difficulties at 4-6 months and at 48 months in a study combining birth cohorts from two different countries (Oliveira et al., 2015).

To explore comorbidity and feeding difficulties further, a Belgian study of children referred to a feeding clinic characterised participants based on their pathophysiology. The authors reported that only a small minority of children did not have an underlying diagnosis: 86% had a medical disorder and 18.1% had a behavioural problem (Rommel et al., 2003). Amongst the medical conditions, gastrointestinal problems were most frequent (51%), with gastro oesophageal reflux disease diagnosed in a third of the total study population. Twenty-five children had food allergy (3.5%), although the study did not specify what type of food allergy or how it was diagnosed. Other medical conditions were neurological (20%), genetic (9.2%), ear nose and throat (5.7%), cardiac (4.4%) or respiratory (4.4%) in nature. The majority of children with oral feeding problems were under two years of age, with a decrease in feeding difficulties seen at ages 3-4 years, attributed to spontaneous improvement of gastro oesophageal reflux disease. This was a large study, with seven hundred participants, however it is likely to be a skewed representation of feeding difficulties as the research took place in a tertiary hospital clinic.

3.11.6 Feeding difficulties in children with food allergy

The management of behavioural feeding problems in food allergy was highlighted as a training need in a study of dietitians from the UK, Australia and USA, suggesting it is a very widespread issue (Maslin et al., 2014). In food allergic infants, it has been hypothesised that feeding dysfunction may persist after allergens are removed from the diet, secondary to learned associations with food and discomfort and reinforcement of maladaptive feeding behaviours. In addition, the food allergic child may have reduced opportunities to participate in typical social eating norms (Haas, 2010). Studies that have investigated the incidence of feeding difficulties in food allergic children, have mainly focused on children presenting with non-IgE mediated

gastrointestinal symptoms and most have been hindered by a lack of a comparative control group and absence of nutritional data (Meyer, Rommel, et al., 2014; Mukkada et al., 2010; Pentiuk, Miller, & Kaul, 2007; Wu, Franciosi, Rothenberg, & Hommel, 2012).

In a small study Pentiuk et al. (2007) described fifteen children with eosinophilic oesophagitis who presented with food refusal, oral aversion and vomiting. Seven had a positive SPT to at least one food allergen, although the majority of the children also had neurological/developmental problems, which were highly likely to be implicated in their feeding difficulties. Similarly, Mukkada et al. (2010) undertook a retrospective medical record analysis of 200 children with eosinophilic disease. Thirty-three had significant feeding dysfunction, of which 88% had diagnosed food allergy, however the measurement of feeding dysfunction used a non-validated protocol. A UK study of 437 children diagnosed with food allergies affecting the gastrointestinal tract reported that aversive feeding occurred in 30% of the sample (Meyer, Rommel, et al., 2014). However a limitation of the study was that information was collected retrospectively. A study by Wu et al. (2012), was one of the few studies in the literature to have recruited a control group, reporting that children with gastrointestinal food allergy have a significantly higher number and frequency of behavioural feeding problems compared to healthy children.

3.12 Food Neophobia

3.12.1 Definition and presentation of food neophobia

Food neophobia, meaning “a fear of new food”, commonly presents in children as a reluctance to eat unfamiliar foods. It increases rapidly once infants become more mobile and independent, peaking between the ages of two to six years (Addessi et al., 2005). It is a normal phenomenon, which is thought to provide a protective benefit by preventing ingestion of potentially toxic foods. Food neophobia and fussy eating are often confused, however food neophobia is in fact a subset of fussy eating (Dovey et al., 2008). They are related concepts, but are thought to be behaviourally distinct with different factors affecting severity and expression (Galloway, Lee & Birch, 2003). Neophobia does not reflect a fixed dislike, but a transient one that may be altered via subsequent food experience (Birch, 1999).

3.12.2 Prevalence and measurement of food neophobia

Unlike fussy eating and feeding difficulties, the measurement of food neophobia is relatively standardised between studies, with most research in the literature using the Children’s Food Neophobia Scale (CFNS) (Pliner, 1994) (appendix 11). This questionnaire will be described in

detail in chapters four and five. Briefly, the CFNS measures food neophobia on a continuum, rather than a binary yes/no outcome. Although neophobia is defined as one standard deviation above the mean of the study sample (Pliner, 1994), studies tend not to report the results in this manner so it is not possible to report an exact prevalence. Estimations are that 20-30% of children are significantly neophobic, but specific data is not available (Scaglioni et al., 2011; Wardle & Cooke, 2008)**.**

Unlike fussy eating, which is more common in boys, no difference between gender has been reported in a wide range of children from preschool to adolescents (Cassells, Magarey, Daniels, & Mallan, 2014; Cooke, Carnell, & Wardle, 2006; Falciglia, Couch, Gribble, Pabst, & Frank, 2000; Mustonen, Oerlemans, & Tuorila, 2012; Russell & Worsley, 2008). Evidence suggests that neophobia may be genetic in nature. A twin study of 8-11 year olds (n = 5390 pairs) reported that 78% of the variation in neophobia scores is due to heritability (Cooke, Haworth, & Wardle, 2007). Another study reported that girls food neophobia levels were positively associated with maternal, but not fathers' levels (Galloway et al., 2003).

3.12.3 Food neophobia, nutritional intake and growth

Overall it can be said that the effect of food neophobia on dietary consumption is similar to that of fussy eating, with lower intakes of vegetables and meat reported by studies, but a lack of detailed nutritional information provided. For example, an Australian study of two year old children (n = 330) reported that neophobia was negatively associated with fruit and vegetable variety, but positively associated with intake of discretionary foods (i.e. high fat foods with poor nutritional value) (Perry et al., 2015). Food neophobia was not found to be related to weight and the study did not report nutritional intake data. The authors speculated that food neophobia and BMI might have a curvilinear relationship (i.e. be related to both under and over weight) yet the sample size was not large enough to detect this association.

Similarly, a study of 564 two to six year old children in London reported a negative association between neophobia and vegetables, fruit and meat, but no association to sweet/fatty snack foods, starchy carbohydrate foods or eggs (Cooke, Wardle, & Gibson, 2003). Likewise, Russell et al. (2008) reported food neophobia was associated with reduced preference for all food groups in preschool children and less healthful food preferences, but did not report any nutritional information. In a rare study that used mealtime observation and direct recording of food consumption rather than parental report, 4-5 year olds (n = 109) took part in three test lunch meals where they were presented with a number of food items including chicken, cheese, bread, chocolate biscuits and grapes and carrots. Neophobia was associated

with lower consumption of fruit and vegetables, protein foods and total calories, but no association with starchy or snack foods (Cooke et al., 2006). This implies children with food neophobia don't compensate for a lower intake of fruit and vegetables by eating more of foods in other categories.

In older children, a study of nine year olds in the US (n = 70) reported no difference in nutritional intake by neophobic status, with the exception of vitamin E. However, neophobic children were rated as having a lower quality diet overall due to the lack of food variety and higher intake of saturated fat (Falciglia et al., 2000). In a Finnish study, 8-11 year old children with neophobia reported lower preference for cheese, fruit, vegetables, fish, meat and ethnic/exotic foods (Mustonen et al., 2012) demonstrating that dislikes of neophobic children are broadly similar across countries.

3.12.4 Food neophobia and food allergy

No studies were identified that assess food neophobia in children currently consuming an exclusion diet for food allergy. One study was identified that investigated food neophobia in children with previous food allergy. A study of French children compared food neophobia levels in children who had outgrown a food allergy to that of a sibling who had never had a food allergy (Rigal, Reiter, Morice, De Boissieu, & Dupont, 2005). The study demonstrated that following an exclusion diet for both single and multiple food allergy results in higher food neophobia. Food neophobia was worse if the diagnosis of food allergy had been delayed. This was attributed to children being reluctant to try new foods if they have had experience of several adverse reactions in the past. Although informative, this study did not assess dietary intake, so it is not possible to say whether the higher rates of food neophobia impacted on nutritional intake.

3.13 Food preferences

3.13.1 Overview of food preferences

Foods preferences in children are a good predictor of their self-selected intake, with experimental studies showing correlations of 0.6-0.8 (Birch, 1979). As there is an innate preference for high caloric foods, both children and adults prefer and tend to choose energy dense foods that are satiating (Scaglioni et al., 2011). Children's food preferences are therefore generally not consistent with a healthy diet, with fatty and sugar foods rated most highly across age and gender (Cooke & Wardle, 2005).

Preference for foods is due to a combination of several properties (e.g. taste, olfaction, texture, presentation and temperature). All senses play a role in influencing dietary preference and choice, rather than just the taste e.g. the influence of foul odour, strange colour or a negative association with an experience will influence preference for a food. This is confirmed by a study of 4-5 year old children showing that foods were not preferred due to one simple sensory property (e.g. sweetness, saltiness or creaminess) (Wardle, Sanderson, Leigh Gibson, & Rapoport, 2001). As previously discussed, children's food preferences can also be influenced by parenting practices.

3.13.2 Measurement of food preferences

Food preferences are measured either directly in a laboratory setting where food consumption and choice is measured, or indirectly using either a paper based or computerised questionnaire. Questionnaires are composed of a list of foods, usually divided into categories, derived from FFQs, or may focus on a particular subgroup of foods (e.g. snack foods or fruit and vegetables). Questionnaires may be completed by the child or parent, depending on the age of the child and the complexity of the questionnaire.

There is disagreement whether maternal report of child food preferences is accurate and/or reliable. Correlations between children's food preferences at age eight years and maternal reports were highly correlated in a study investigating liking for 90 different foods (Skinner, Carruth, Bounds, & Ziegler, 2002). Other studies assessing liking for specific food groups have shown only moderate agreement for fruit and vegetables (Vereecken, Vandervorst, Nicklas, Covents, & Maes, 2010). In a study of ice cream flavour preference in children aged 3-10 years, only 39% of mothers were able to correctly predict their child's most preferred flavour, but significantly more (61%) were able to predict the least preferred flavour (Liem, Zandstra, & Thomas, 2010). This may be due to the fast rate at which children change their food preferences and the fact that liking for ice cream is very skewed. Where possible, measurement of children's food preferences should be obtained directly rather than via proxy parental report.

The choice of method to assess food preference will depend largely on the research question and resources available. Laboratory testing of food preference is labour intensive and may not reflect real world choices due to the artificial environment. Sensory testing in children is difficult due to differences in cognitive stage and ability to understand the task in hand, therefore the test must match the child's stage of development (Liem & Zandstra, 2010). School aged children, unlike preschool children, have reasoning ability, memory and language skills

to allow for more complex tests (Popper & Kroll, 2005). Additionally, children need to feel motivated to take part in sensory testing and feel confident they are doing well at task to maintain attention (Liem & Zandstra, 2010). Conversely there are also limitations of questionnaires rather than sensory testing with real foods. Questionnaires are an indirect measure that may be subject to social desirability bias. Different methods of measuring food preferences will be compared and discussed in more detail in chapter five.

3.13.3 Factors that influence children's food preferences

Many factors (e.g. genetic, environmental cultural and social environment) can mediate the acceptance or rejection of foods (Scaglioni et al., 2011). Culture dictates to a large extent the foods that a child is exposed to and beliefs about appropriate contexts to eat certain foods (e.g. it is normal to eat fish for breakfast in Scandinavia, or rice for breakfast in Japan, but would be considered unusual in the UK) (Wardle & Cooke, 2008). Despite this, children's food preferences are remarkably similar across cultures and countries. In general sweet and fatty foods are the most liked foods by children, with vegetables the most poorly rated (Cooke & Wardle, 2005; Diehl, 1999; Nu, MacLeod, & Barthelemy, 1996; Skinner et al., 2002). Staples such as bread or rice are rarely disliked. A summary of studies of children's food preferences is shown in Table 3.4.

Overall previous research has found no difference in food preference by socioeconomic status, region, parental education or weight status (Diehl, 1999; Nicklaus, Boggio, Chabanet, & Issanchou, 2004; Nu et al., 1996). Gender differences have been reported by a number of studies, with boys demonstrating significantly stronger preferences for fast food/fatty foods, meat and fish, whereas girls report higher preferences for fruit and vegetables (Caine-Bish & Scheule, 2009; Cooke & Wardle, 2005; Diehl, 1999; Nicklaus et al., 2004). It has been suggested that gender differences may occur because girls' food likes are most influenced by healthy eating whereas boys' food likes are more influenced by the satiation and availability of foods (Nu et al., 1996). Perez-Rodrigo et al. (2003) did not detect any gender difference although the study looked at a narrow range of foods only.

Table 3.4 Summary of studies from different countries investigating children's food preferences

Authors, year and country	**N**	**Age**	**Method**	**Outcomes**
Ton Nu et al. (1996) France	222	10-20 year old	List 10 most and least liked foods/ beverages	Most liked foods: pasta, chocolate, coca cola, French fries, ice cream, cakes, pizza, pastries, sweets. Most disliked: offal, chicory, spinach, cabbage, fish, alcohol, cheese, mushrooms, coffee. There were twice as many likes quoted as dislikes.
Skinner et al. (1998) USA	118	28-36 months.	Questionnaire of 196 foods	Children had been offered 77.8% of foods and liked 81.1% foods. Corn, mashed potato and French fries were the only uniformly liked vegetables. French fries were the only liked and eaten food by 100%. 21 of 22 of the most disliked foods were vegetables
Diehl (1999) Germany	1233	10-14 years old	Questionnaire of 114 foods and 14 drinks	Most liked foods: pizza, ice cream, spaghetti, French fries, hamburgers, pudding, corn flakes, potato chips and popcorn. Fast food, candy and salty snacks were the most liked categories. Least liked: liver, canned/steamed fish, raw sauerkraut, red cabbage.
Wardle et al. (2001) UK	215 twin pairs	4 year old	Questionnaire of 93 foods	Mean number of tried foods: 83. Mean number of disliked foods: 6. Most liked: chocolate, biscuits, crisps, ice cream, ice lolly, cake, chips. Least liked: onion, cabbage, cauliflower, green beans, baked beans.
Skinner et al. (2002) USA	70	2 year olds and mothers.	Questionnaire 194 items (mothers), 90 items (child)	Aged 2-3 years: liked 118 foods, disliked 22 foods, never tried 54 foods. Aged 8 years: liked 125 foods, disliked 33 foods, never tried 37 foods. Most liked foods: fizzy drinks, popcorn, white roll, French fries, crisps, cookies, pizza.17 of 24 most disliked foods were vegetables.
Perez-Rodrigo et al. (2003) Spain	3534	2-24 year old	Asked to rank most liked foods.	Most liked foods: pasta, rice and meat. Least liked foods: vegetables, legumes and fish. 47% disliked all vegetables. Sweet/fatty foods not assessed.
Cooke & Wardle (2005) UK	1291	4-16 years old	Questionnaire of 115 foods	Children had tried 98/115 foods. Most liked foods: chocolate, pizza, ice cream, pasta, strawberries, ice lolly. Least liked foods: spinach, leeks, marrow, swede, sprouts, turnip, liver. Fatty and sugary foods were the most well-liked category.

3.13.3.1 Tracking of food preferences and the effect of age

Longitudinal studies have investigated whether food preferences in infancy are predictive of food preferences in later life. As food preference is a function of familiarity, it would be expected that as a child grows older they would be exposed to and become familiar with a greater variety of foods, thereby develop liking for more foods. However a longitudinal study found that the number of liked foods only increased by 3% between the ages of two to eight years (Skinner et al., 2002), thus highlighting the importance of establishing a varied diet in early childhood. It is possible that six years was not long enough to see an increase in food preferences in this study. It may be that a widening of food repertoire occurs after puberty, potentially due to a reduction in neophobia and increase in autonomy around this age and opportunity to eat away from home or the family (Nu et al., 1996).

Studies that have investigated food preference extending into early adolescence and adulthood have demonstrated relative stability. A study of 5-11 year old girls reported that liking for ten palatable snack foods was modestly stable over the study period with food items generally becoming more liked over a six year period (Rollins, Loken, & Birch, 2010). The study included items such as ice cream, cookies, pretzels and popcorn, therefore it is unknown if the findings extrapolate to other foods. A French study that compared foods eaten at nursery at age two years to preferences up to eighteen years later reported early preference was the most contributing factor in predicting later preferences (Nicklaus et al., 2004). The association was particularly evident for mature cheeses and other strongly flavoured foods, suggesting early taste preference may be important.

3.13.3.2 Heritability of food preferences

Evidence suggests that food preferences may be heritable to some extent, although it is not known what exactly is inherited; innate preference for bitter taste or temperament towards food (Breen, Plomin, & Wardle, 2006). Research investigating the concordance of food preferences between parent and child is contradictory. A meta-analysis of studies of parent– child pairs conducted in 1993 showed that there were significant, but small, parent–child correlations for food preferences (Borahgiddens & Falciglia, 1993). An overall effect size of 0.17 was reported, with the same effect seen for mothers and fathers, however only five studies had sufficient data to be included. This finding was attributed partly to the age difference between children and their parents. Because preferences may change over the lifespan, weak correlations between children and adults could underestimate the familial association. A later study by Skinner et al. (1998) of children aged 28-36 months (n = 118) siblings and parents demonstrated a strong

concordance of 82-83% in food preferences between family members. The most limiting factor related to food preferences were foods that were never offered to the child. No one family member (mother, father or sibling) was more influential than the other on the index child's food preferences.

Following on from this, two twin studies undertaken in a UK population suggest that heritability of food preferences is dependent on the food group. Breen and Plomin (2006) in a study of 4-5 year old twins demonstrated a substantial genetic effect for liking of protein foods (0.78), moderate genetic effects for liking of vegetables (0.37) and fruit (0.51), and a small genetic effect for liking of dessert foods (0.20). A later larger study of a UK cohort of twins aged three years (n = 2686) assessed preference for 114 foods using a parental report questionnaire (Fildes et al., 2014). Genetic effects dominated for fruit, vegetables and protein rich foods (48-54%), whereas shared environment effects dominated for snacks, dairy and starchy foods (54-60%). The authors concluded that the home environment is the main determinant for children's liking for energy-dense food and that parents are correct in perceiving a moderately strong genetic component for commonly rejected foods such as vegetables, whereas health professionals are correct in viewing the home environment as highly influential in children's liking for energy-dense snacks and starchy foods.

3.13.4 Food preferences and food allergy

A literature search identified no studies that had been undertaken of food preferences in food allergic children, demonstrating that this is an under researched area. As food preferences are dependent on exposure and familiarity and the management of food allergy necessitates an exclusion of a food (or food group); it provides an interesting scenario to investigate. Two studies that have investigated food preference of children aged 4-7 years old who had consumed soya or EH casein formula were identified (Liem & Mennella, 2002; Mennella & Beauchamp, 2002). Those who were fed a hydrolysate or soya formula had higher preference for sour and bitter flavoured juices, and were more likely to like broccoli (Mennella & Beauchamp, 2002). However no association was found between consumption of an EH casein or soya formula and sweetened juice or liking for sugary foods (Liem & Mennella, 2002). No data is provided on the reasons and clinical history of children who were fed the substitute formula or if they excluded dairy products. Eighteen per cent of children in the soya or EH casein formula group were noted to currently have CMA, therefore it is probable that the majority did not exclude dairy foods, so the study's findings are not necessarily generalisable to children with CMA.

3.14 Dietary Variety

Humans are omnivores with adaptable dentition and digestive systems, allowing consumption of a wide variety of foods (Wardle & Cooke, 2008). 'Dietary variety', synonymous with 'dietary diversity' or 'food diversity', is defined as the number of different foods or food groups consumed over a given reference period. In theory, consumption of a varied diet should reduce the risk of developing a deficiency or excess of any particular nutrient. Therefore the consensus is that dietary variety and dietary quality are positively correlated i.e. the greater the number of food that are consumed, the more nutritious and balanced the diet will be (Ruel, 2003).

A limitation of dietary variety is that it is focused on nutritional adequacy and does not necessarily take into account excess consumption. As dietary variety tends to measure nutritious foods, high fat and high sugar foods may be excluded (Cox, Skinner, Carruth, Moran, & Houck, 1997), although some research has separated food groups into "core" and "non-core" foods (Scott et al., 2012). It is possible to consume a limited number of nutrient dense foods and have a narrow dietary variety. Conversely it is possible to consume several different foods of low nutritional quality and have a high dietary variety (Cox et al., 1997). However despite these limitations dietary variety has been shown to correlate strongly with dietary adequacy in toddlers ($r = 0.74$) (Cox et al., 1997). Furthermore, in a study of children aged 1-8 years old, a strong relationship was reported between dietary diversity and indicators of child growth, in addition to it being a simple and quick indicator of the micronutrient adequacy of the diet (Steyn, Nel, Nantel, Kennedy, & Labadarios, 2006).

3.14.1 Measurement of dietary variety

Dietary variety is usually measured using a simple count of foods or food groups (Ruel, 2003) using either a 24 hour dietary recall, a food diary or a FFQ approach. A number of different food groupings, classification systems and reference periods have been used, meaning a lack of consistent approach. It is recommended that food groupings should be based on the dietary patterns of the specific age groups and the contribution of particular staple foods to nutrient adequacy (Ruel, 2003). Therefore for infants, measurement of dietary diversity should ideally take into account age-appropriate weaning foods. As dietary variety in children peaks steeply in the first three days and tends to taper between 10-15 days, it is recommended that a reference period of at least three days is used in order to capture typical consumption patterns (Falciglia, Horner, Liang, Couch, & Levin, 2009), therefore a one day 24 hour recall may not provide a realistic reflection of intake.

It is also questioned whether portion size should be recorded when measuring dietary variety (Ruel, 2003). It is arguable whether mere exposure to a food is significant, versus consuming a meaningful amount of a food sufficient to make a nutrient contribution (Scott et al., 2012). This is particularly relevant in assessing dietary intake in infants and toddlers, where portions may be very small and food may be refused after a couple of spoons. One approach that has been used is to record the food if half an age appropriate portion is consumed (Cox et al., 1997). Methods for measuring dietary variety will be considered further in chapter four.

3.14.2 Dietary variety and food allergy

Recently there has been considerable interest in the effect of variety of the infant diet in the prevention of atopic disease. It is hypothesised that exposure of the infant gut to different food antigens might influence the development of immune tolerance (Roduit et al., 2014). Two notable publications investigating the diversity of the infant diet and risk of later allergy using prospective birth cohort data have been published recently (Nwaru et al., 2014; Roduit et al., 2014).

Roduit et al. (2014) recruited 856 children from rural communities in five European countries. Food diversity scores were calculated based on the number of different foods introduced and included in child's diet up to the age of one year. Increased diversity of food within the first year of life was found to have a protective effect on asthma, food allergy and food sensitisation in the first six years of life. This inverse relationship persisted when children with food allergy were excluded, thus limiting the bias of reverse causality (i.e. participants who have symptoms or a high risk of food allergy may limit or delay introduction of certain foods). However, this study only looked at twelve different food/food categories (milk, yogurt, other milk products, eggs, nuts, vegetables or fruits, cereals, bread, fish, soy, margarine, butter, cake and chocolate).

Nwaru et al. (2014) (n = 3142) reported similar findings in a Finnish population. Food diversity, again defined as the number of complementary foods introduced at specified time points up to the age of one year, was inversely associated with risk of asthma, wheeze and allergic rhinitis. This was hypothesised to be due to the diversity of intestinal microbiota, although gut microbial composition was not measured. The foods included in the diversity calculation were slightly different to Roduit et al. (2014) (cows' milk and formula, potatoes, carrots, turnip fruit and berries, cereals, other cereals, meat, fish, egg, cabbage spinach and lettuce) and the authors acknowledge that the definition of food diversity might be enhanced by looking at a wider spectrum of the infants diet.

Unfortunately neither of these two studies differentiated between home made and commercially produced infant foods. This is important as there is debate whether commercially produced infant food increases or decreases infant food variety (Hurley & Black, 2010; Mesch et al., 2014). Of note, data from a UK birth cohort suggests that an infant diet high in fruit, vegetables and home prepared foods, with only occasional use of commercially produced infant food, is associated with less food allergy at age two years (Grimshaw et al., 2014). However the authors did not rule out that increased consumption of home prepared foods a *result* of food allergy (i.e. being unable to access allergen free readymade baby food), rather than the cause of the food allergy. The use of commercial readymade baby food will be discussed further in chapter four.

To date, no published research has specifically investigated the dietary variety in children consuming an exclusion diet for food allergy. It appears logical that children prescribed an exclusion diet will have a less varied diet as they are limiting a whole food or food group. Paradoxically, it may be that parents of children consuming exclusion diets are forced to widen their normal food patterns to include alternative foods and recipes, potentially resulting in a broader variety of foods consumed. However, this is speculation. It is not known if this is the case in children current or previously following an exclusion diet.

3.15 Summary of literature review on infant and child eating behaviour

Infant and child food preferences and eating behaviour are complex in nature. They are influenced by a number of factors, which may occur in utero, during milk or solid feeding. Feeding behaviour may be affected positively or negatively at any of these stages. Both genetic and environmental factors, such as parental feeding practices, are important. This complex interaction was summarised at the beginning of this chapter in Figure 3.1.

Problems such as fussy eating, feeding difficulties and food neophobia are relatively common in healthy developing children, although it is difficult to measure their prevalence accurately due to differences in methodology. They may negatively influence dietary variety, nutritional intake and growth, although evidence is inconsistent. Dietary variety is of particular interest in the development of allergy. Little is known about the rates of fussy eating, feeding difficulties or food neophobia in food allergy or if there is an effect of substitute formula and dietary restriction on food preference and dietary variety in the short or long term.

4 Chapter 4: Study one

4.1 Overview

This chapter examines the eating habits of a group of infants consuming a Cows' Milk Exclusion (CME) diet, compared to a control group of infants consuming an unrestricted diet. Parents of both groups completed a number of validated questionnaires; specifically measuring fussy eating, feeding difficulties, food neophobia and dietary variety. The questionnaires used will be described and their use justified. The results are discussed in relation the management of children with Cows' Milk Allergy (CMA) and any clinical implications the findings may have.

4.2 Background

4.2.1 Rationale

CMA is known to affect ~1.26-2.9 % of children in the UK (Schoemaker et al., 2015; Venter et al., 2008). Some of these children may be allergic to multiple foods or food groups. It is known that parents may incorrectly perceive their child to have a food allergy (Venter et al., 2006) and that CME diets are sometimes initiated unnecessarily (Eggesbø, Botten, & Stigum, 2001; Sinagra et al., 2007), meaning greater than 3% of children are likely to be following a CME diet. In practice this means that many children are excluding a major food group from their diet at a time in life that is critical for growth, development and establishment of eating habits. Infants with CMA who are not exclusively breastfed are prescribed substitute infant formula, which have an altered taste. Parents are also advised that their child should follow a special weaning diet avoiding all forms of cows' milk, usually until at least one year of age, but this may be required to continue for much longer (Spergel, 2013).

Fussy eating and food neophobia are very common in young children (Dovey et al., 2008). Up to 20% of infants and toddlers in the UK are reported to be "problem" eaters by their parents with some studies reporting up to 50% are fussy eaters (Carruth, Ziegler, Gordon, & Barr, 2004). However, this tends to be a transient phase that does not generally have a detrimental effect on growth (Wright et al., 2007). In healthy infants and toddlers, it is known that development of feeding skills and behaviours occurs over a wide age range and is influenced by many factors (Carruth & Skinner, 2002). Feeding difficulties (which includes refusal of certain textures, retching and gagging on food, extremely prolonged mealtimes) are known to be more common in certain medical conditions (e.g. autism spectrum disorder, cerebral palsy) (Levy et al., 2009).

In a young child with suspected or confirmed food allergy, where at least one food group is already being restricted, fussy eating and feeding difficulties are likely to have a considerable negative impact on eating habits and food intake. Conversely, it may be that consuming a substitute infant formula and a CME diet has a *positive* effect on fussy eating; leading children to develop taste preferences for bitter tasting fruits and vegetables (Mennella & Beauchamp, 2002). Indeed, as milk and milk powder are ubiquitous in many confectionary and snack foods (e.g. chocolate and biscuits), it is possible that infants and toddlers consuming a CME diet may adopt a "healthier" eating pattern. Anecdotally, it has been reported by parents of children consuming exclusion diets that they use less processed foods and prepare meals using fresh ingredients and alternative products, forcing them to widen their family's food repertoire, thus resulting in a *more* varied diet for the child.

To date there has been limited research directly investigating the prevalence of eating problems in children consuming a special diet for food allergy (Haas, 2010). The existing studies (Meyer et al., 2014; Mukkada et al., 2010; Pentiuk, Miller, & Kaul, 2007; Wu, Franciosi, Rothenberg, & Hommel, 2012) have all focused on non-IgE food allergy and most have not included a control group of children consuming an unrestricted diet. This study will provide both direct (food records) and indirect (questionnaires) measures of fussy eating in infants and toddlers. Results of this study will inform dietitians, allergy nurses and doctors of the degree of eating problems in children following a special diet for food allergy. It aims to show whether levels of fussy eating, food neophobia and feeding difficulties are higher in infants consuming a CME diet, compared to infants consuming a normal diet. If this is demonstrated, it will provide further evidence for providing sufficient allergy services in the UK, ensuring children are diagnosed in time and reviewed appropriately. Conversely, if those following a CME diet are found to have lower levels of fussy eating and greater dietary variety, this provides reassurance to health care professionals and parents alike.

4.2.2 Aim and objectives

The overall aim of this study was to investigate eating habits in infants and toddlers consuming a CME diet.

The primary objectives of the study were:

- To determine the degree of fussy eating, feeding difficulties and food neophobia in infants and toddlers consuming a CME diet compared to a control group.
- To determine dietary variety in infants and toddlers consuming a CME diet compared to a control group.

A secondary objective of the study was:

- To determine the growth status of young children consuming a CME diet, compared to a control group.

This was set as a secondary objective as the measurement of growth in children consuming a CME diet has already been described extensively by previous studies (Agostoni et al., 2007; Isolauri et al., 1998; Seppo et al., 2005; Vieira et al., 2010). In addition, it was not realistic to recruit a sufficiently powered sample size within the allocated resources and timeframe to evaluate the effect of a CME diet on growth.

4.3 Justification for choice of questionnaires

An extensive literature search indicated that no one unique tool is suitable for assessing all aspects of infant and toddler eating habits. This was supported by two recent published reviews (de Lauzon-Guillain et al., 2012; Vaughn, Tabak, Bryant, & Ward, 2013). Therefore, four validated parental report questionnaire measures were selected and used, in addition to one other questionnaire that was constructed for the purpose of this study. Overall, questionnaires were chosen on the basis that they had previously been validated in the age group in question and could be completed in a reasonable timeframe. The following section provides detailed descriptions of these questionnaires and justification for why they were used.

4.3.1 Fussy eating

As outlined in the previous chapter, fussy eating is generally defined as consuming "a limited variety of foods" (Dovey et al., 2008; Taylor, Wernimont, Northstone, & Emmett, 2015). A number of fussy eating questionnaires were identified in the literature. After considering the advantages and disadvantages of each questionnaire, the Picky Eater questionnaire (Carruth et al., 1998) (appendix 10) was chosen for this study.

Carruth's questionnaire is a parent-report measure consisting of 10 items describing specific behaviours related to fussy eating. All items are measured using a seven point likert scale. Each question has anchor descriptors specific to that question (e.g. from "not at all" to "extremely" or from "never" to "always"). Five questions are reverse scored. The questionnaire was adapted from a longer 20-item questionnaire (Pliner & Pelchat, 1986). It has been validated against two types of dietary records and found to have good reliability in children aged 24-36 months old. In the validation study, 10 items of the questionnaire were found to differentiate picky eaters from non-picky eaters. More recently the 10-item questionnaire has been validated against behavioural measures of eating in 12-month old infants (Blossfeld et al., 2007), where the questionnaire was divided into two subscales: "pickiness" (seven items) and "new foods"

(three items). Scores are computed giving a minimum score of 10 and maximum of 70, with a higher score indicating a higher level of fussy eating. In the current study the Cronbach alpha coefficient was 0.859, demonstrating good internal consistency.

Fussy eating can be difficult to quantify accurately and is often evaluated by a parental report tool or asking of a single yes/no question, rather than analysis of dietary records (Bandini et al., 2010). Although several tools have been developed for measurement of preschool children's fussy eating behaviour, none have been specifically designed for children under 18 months old and this was identified as a gap in the literature in a recent review (de Lauzon-Guillain et al., 2012). The only study identified that assessed levels of fussy eating in infants under nine months old, was where parents were asked to simply rate their child as "not a picky eater", "somewhat a picky eater" or a "very picky eater" (Carruth et al., 2004).

Wright et al.'s study (2007) of fussy eating in 445 children from the millennium birth cohort study in the North of England is frequently cited. However the mean age of the participants in that study was 30 months. Their questionnaire was constructed for the purpose of the study and was therefore not validated. Likewise the questionnaire used in the ALSPAC cohort study (Northstone et al., 2001) that has since been used in other large birth cohort studies (Dubois et al., 2007), was also constructed for the purpose of that study and is also not validated. Finally, the Children's' Eating Behaviour Questionnaire (CEBQ) (Wardle, Guthrie, et al., 2001) (appendix 9) was deemed to be one of the few questionnaires to achieve all validation criteria, with demonstrable internal consistency, reliability and construct validity (de Lauzon-Guillain et al., 2012), however it has only been validated in children aged three years and older. The CEBQ was used in study two of this PhD and will be discussed in more detail in chapter five.

4.3.1.1 Other questionnaires that partially address fussy eating

There are several validated questionnaires available that assess infant and toddler diet with an emphasis on healthy eating and obesity (e.g. the Infant Feeding Questionnaire and Preschool Feeding Questionnaire) (Baughcum et al., 2001). Although these tools do include some questions that address fussy eating, their primary aim is to assess the effect of infant feeding practices on the development of overweight and obesity, therefore they were not thought to be suitable for this study. Similarly questionnaires that focus primarily on parental management of feeding or obesity (e.g. the Child Feeding Questionnaire) (Birch et al., 2001), were also not appropriate, nor were methods that focus on fussy eating as a component of childhood anxiety (Zucker et al., 2015).

4.3.2 Food Neophobia

Food neophobia is defined as a reluctance to try new and unfamiliar foods. The most commonly used questionnaire for food neophobia is the Child Food Neophobia Scale (CFNS) (Pliner, 1994) (appendix 11), which was chosen for this study. This questionnaire has ten items, five of which are reversed scored and a seven point likert scale from “strongly agree” to “strongly disagree”. The scores are summed to give a food neophobia score ranging from 10-70, with a higher score indicating a higher level of food neophobia.

The original Food Neophobia Scale (FNS) was developed for use in adults (Pliner & Hobden, 1992) and was found to successfully predict behavior in willingness to consume novel foods, with good test retest reliability and internal consistency. It was later adapted to be a parental-report questionnaire for 5-11 year old children in Canada (Pliner, 1994). Using this tool, it was shown that parental reports of children’s food neophobia were related to children’s willingness to try novel food in a laboratory context. It has also been used in studies of children in the UK (Cooke, Haworth, & Wardle, 2007; Cooke, Carnell, & Wardle, 2006). Good internal consistency of the 10-item questionnaire as a parent report questionnaire has been reported with a Cronbach alpha coefficient of 0.91 indicated in a study of preschool children (Russell & Worsley, 2008). In the current study the Cronbach alpha coefficient was 0.905.

In order to remove items that may not be relevant to younger participants, a shorter six-item version of the CFNS has been developed for use in 2-6 year old British children (Cooke et al., 2006). The following items were removed in the six-item version of the questionnaire: *My child likes foods from different cultures. For my child, food from cultures different to her own looks too weird for her to eat. At social gatherings, my child will try a new food. My child likes going places serving foods from cultures different to her own*. This six-item version has been shown to have high internal consistency (Cronbach’s alpha of 0.92) and has recently been used in a cohort of two-year old children in Australia (Cassells et al., 2014). Subsequently the CFNS has been further reduced to a four-item questionnaire, using the questionnaire items that focus most on new, rather than unfamiliar, foods in a study of 8-11 year old twins (Cooke et al., 2007). Both the four item and six item versions are measured on a four-point scale from "strongly disagree" to "strongly agree", rather than a 1-7 likert scale used in the original CFNS. The full 10-item questionnaire can still be used in younger children and has been used in a study of Australian children aged 2-5 years old, the only adaptation being the removal of word “ethnic” (Russell & Worsley, 2008).

Few studies have used the CFNS in children under two years of age, probably due to the fact that characteristically food neophobia peaks between the ages of 2-6 years old

(Addessi, Galloway, Visalberghi, & Birch, 2005). In this study the participants were aged between 8-30 months, as this is the age range during which CMA typically occurs. Rather than use the shortened four- or six-item questionnaire, it was decided to use the original full 10-item questionnaire, as this was also being used in study two of older children in this PhD . Using the same questionnaire in both studies would allow direct comparison of scores in two cross sectional groups from the same geographical area: infant/toddlers who are currently consuming a CME diet and older children who previously consumed a CME diet.

4.3.2.1 Other food neophobia measures

Other measures of food neophobia exist, namely The Food Attitude Scale (FAS) (Raudenbush, van der Klaauw, & Frank, 1995), which has not been adapted for use in young children and the Food Situations Questionnaire (FSQ) (Loewen & Pliner, 2000) which is a self-report measure, therefore requiring children to be able to read and write. Neither of these measures was suitable in this age group and they will be discussed further in chapter five.

4.3.3 Feeding Difficulties

As previously outlined, feeding difficulties refers to a spectrum of problematic eating behaviours such as excessive spitting out of food, crying/irritability at feeding time, eating extremely slowly, retching at the sight of bottle or spoon, apparent difficulty in swallowing, throwing and pushing away food (Crist & Napier-Phillips, 2001; Levy et al., 2009; Lewinsohn et al., 2005). After comparing and appraising different methods, the Montreal Children's Hospital Feeding Difficulties questionnaire was chosen for this study (appendix 12) (Ramsay, Martel, Porporino, & Zygmuntowicz, 2011). It is, to the author's knowledge, the only validated questionnaire for use in children under two years of age.

The Montreal questionnaire was developed and validated to identify feeding problems in children aged six months to six years. It is an easy to use measurement that has been demonstrated to be valid and reliable in children with and without medical diagnoses and could be quickly administered in an allergy clinic setting, in approximately five minutes. The questionnaire is comprehensive, covering the following feeding domains: oral motor, oral sensory, appetite, maternal concerns about feeding, mealtime behaviours, maternal strategies used and family reactions to child's feeding. The questionnaire consists of 14 items. Each item is rated on a seven point likert scale, giving a minimum score of 14 and a maximum score of 98. Seven items are negatively scored. A cut off value of a score of 45 is diagnostic of feeding difficulties and is both sensitive and specific (Ramsay et al., 2011). The questionnaire was found to have good reliability and internal consistency, with correlation coefficients for the

individual items ranging from 0.69 to 0.98. In the current study the Cronbach alpha coefficient was 0.862 demonstrating good internal consistency.

When selecting a suitable questionnaire, the priority was to choose a method that was valid, easy to administer, age appropriate and was suitable for a normative and clinical sample. Previous studies have used a range of different methods to assess and identify feeding difficulties/disorders in children. These include objective physical assessment measures (Arvedson, 2008), detailed history taking (Levy et al., 2009), retrospective review of medical notes (Meyer, Rommel, et al., 2014), validated questionnaires (Crist & Napier-Phillips, 2001) and non-validated questionnaires constructed for the purpose of individual studies (Northstone et al., 2001; Wright et al., 2007).

Several validated feeding difficulties questionnaires exist, which all rely on parental report. They include: the Behavioral Pediatrics Feeding Assessment Scale (BPFAS) (Crist & Napier-Phillips, 2001), the Child Eating Behaviour Inventory (CEBI) (Archer, Rosenbaum, & Streiner, 1991) the Feeding Strategy Questionnaire (FSQ) (Berlin, Davies, Silverman, & Rudolph, 2011) and the STEP CHILD questionnaire (Seiverling, Hendy, & Williams, 2011). However none of these measures are wholly age appropriate or suitable for the participants in this study and all are lengthy questionnaires. BFPAS consists of 35 items and has been validated in children nine months to eight years old. CEBI consists of 40 items and has been validated in children aged 2-12 years old. The FSQ again has 40 items and has been validated in 2-6 year olds. The STEP CHILD questionnaire has been adapted from a questionnaire of feeding problems in adults with learning difficulties. It was only validated in five-year old children and has not been validated in a non-clinical sample (Seiverling et al., 2011). On the other end of the age spectrum, the Baby Eating Behaviour Questionnaire (Llewellyn, van Jaarsveld, Johnson, Carnell, & Wardle, 2011) has been developed for infants under six months, to assess feeding skills in infants on predominantly liquid diets. This is not suitable for the participants in this study who are aged 8-30 months old and consuming solids foods.

4.3.3.1 Other measures of feeding difficulties

As feeding difficulties often occur with a complex multifactorial background, it is not always appropriate or advisable to assess feeding in isolation (Dovey et al., 2008). Factors such as oral and tactile sensory sensitivity, child temperament and social interaction may influence the initiation and maintenance of feeding problems. Questionnaires to measure these precipitating factors are plentiful, however as the objective of this study was fundamentally to determine the

prevalence of feeding difficulties, rather than to investigate their cause, it was felt that measuring these issues was outside the scope of this PhD.

4.3.4 Dietary variety

As already outlined, dietary variety, defined as the number of different foods or food groups consumed over a given reference period, is usually measured using a simple count of foods or food groups (Ruel, 2003). On the whole, the consensus is that dietary variety and dietary quality are positively correlated (Ruel, 2003), therefore dietary variety provides a quick surrogate measure of the nutritional quality and balance of food groups in the diet, without the need to complete a food diary.

4.3.4.1 Use of a FFQ to measure dietary variety

In this study, dietary variety was quantified by the number of foods for which "never" is selected using a Food Frequency Questionnaire (FFQ) as per the methodology of Emond et al. (2010). A number of specifically-designed validated infant FFQs are available (Ortiz-Andrellucchi et al., 2009). In this study, an amended version of the Southampton Women's Study FFQ was used (see appendix 13). This particular FFQ was chosen as it was validated in a group of 12-month old infants against a four-day weighed food diary in a geographical population local to this target study population (Marriott et al., 2009).

The original FFQ was semi quantitative (i.e. it asked respondents to report food portion sizes in addition to frequency of intake). It consisted of a list of 76 food and drinks, divided into subcategories. The subcategories were: non milk drinks (10 drinks), readymade baby foods (9 foods), cereal based foods (8 foods), dairy egg and substitute foods (8 foods), meat fish and vegetarian substitute foods (13 foods), fruits (13 foods), vegetables (10 foods) and sweet and miscellaneous foods (15 foods). The frequency of consumption over the previous 28 days of each food and drink are recorded using a multiple response grid. The frequency options were: "never", "1-3/month", "1/week", "2/week", "3/week", "4/week", "5/week", "7/week" and "more than once per day". There was also a free text "additional" foods category for parents to add any unusual or uncommon foods that were eaten more than four times in the previous month. The parent was also asked the type and volume of infant formula, cows' milk or milk substitute the child drank per day and/or the approximate duration of breastfeeds (in minutes) per 24 hours.

The original questionnaire was adapted for this study in two ways. Firstly, the portion size question was removed, as nutritional intake was not being assessed, thereby making the questionnaire easier to complete. Secondly, three items that may be frequently eaten during a

CME diet were added; namely “soya yoghurt”, “soya cheese” and “other milk substitute yoghurt”. Adapting a food list from an existing FFQ is essential in achieving a dietary assessment of acceptable accuracy. A comprehensive food list including alternative foods consumed by infants consuming an exclusion diet would enable a more detailed examination of food intake patterns. Although the variability in infant diets means that the food list cannot be finite, the inclusion of a free text option to add extra foods made some allowance for this.

4.3.4.1.1 Comparison of FFQs with other methods

Various concepts have been used to rate the diversity of children’s diets, including the Food Variety Score (Scott et al., 2012; Steyn et al., 2006), “limited food repertoire” (Bandini et al., 2010), Variety Index for Toddlers (Cox et al., 1997) and Variety Index for Children (Skinner et al., 2002). These scores use either a 24-hour dietary recall, a food diary or a FFQ approach. When choosing which dietary assessment technique to employ, it must be stressed that all methods have inherent flaws, as eating is a complex and dynamic behaviour influence by many factors and subject to bias. There is no universal criteria for choosing a dietary assessment method in children (Livingstone & Robson, 2000). Assessment of dietary intake in infants is complicated by the fact that their dietary habits can change rapidly depending on their stage of development, they may be looked after by other adults and typically may not eat all the food offered to them (Andersen, Lande, Trygg, & Hay, 2004).

The most critical decision when selecting a dietary assessment method is to match the method to the research question i.e. to ensure the most appropriate assessment technique is used for the required purpose, taking into account whether a specific nutrient, food, food group or time frame is the focus. Often a dietary survey is used to answer different types of research questions simultaneously, which can lead to misreporting (Livingstone & Robson, 2000). As the objective was to measure dietary variety rather than to quantify nutritional intake, a FFQ was used. Use of a FFQ provides additional information about *patterns* of eating and diversity of food groups over a longer period of time, than could be provided by a 24-hour recall or four day food diary. Unlike food diaries, which can be laborious to complete and analyse, responses to an FFQ are standardised, meaning data can be more easily entered and analysed. Food diaries were used in part two of this PhD to assess nutritional intake and their use will be discussed further in chapter five.

A disadvantage of FFQs over a food diary or a 24-hour recall is that over estimation of frequency of consumption may occur, particularly for foods perceived as “healthy” options. Indeed it has been reported that a semi quantitative FFQ may overestimate energy intake in

infants by as much as 25%, compared to a food diary method (Andersen et al., 2004). However when measuring what foods items were or weren't eaten the correlation between FFQ and food diary in the same study was good (r = 0.62). The Southampton infant FFQ, used in this study, was also found to overestimate dietary intake compared to a food diary in one year old infants, however it provided a reasonable to good *ranking* of intake (Marriott et al., 2009). A later research article from the Southampton Women's Study reported very good correlation between their FFQ and a two day food diary (coefficient of 0.72) when assessing the quality and pattern of diet in three year old children (Jarman et al., 2014). In support of this, a systematic review examining the use of FFQs in infants and toddlers concluded that they are an appropriate measure for this age group (Ortiz-Andrellucchi et al., 2009). As with many assessment methods, accurate reporting relies on respondent memory, so recall bias may exist, particularly as a surrogate respondent (parent) is used.

4.3.5 Infant feeding questionnaire

A questionnaire was constructed to collect information on social demographics, family history of allergy, allergic history, infant feeding, relevant medical history and growth history of participants (appendix 14). This enabled information on potential confounding variables to be documented.

The socio-demographic section of the questionnaire included questions on age of participant, gender, ethnic origin, maternal age, parental occupational status and parental educational level. The family history of allergy section asked whether either parent of the participant or any sibling had ever had symptoms of asthma, hay fever, eczema or food allergy. The allergy history section asked whether the participant had ever had symptoms of asthma, hay fever, eczema or food allergy. The food allergy section asked which foods had been excluded, at what age, why and when they had been introduced. These questions were adapted from the International Study of Asthma and Allergies in Childhood (ISAAC) (Asher et al., 1995) questionnaire, with additional included about questions about vomiting, diarrhoea, constipation, colic and abdominal distension. The infant feeding section asked about breastfeeding, use of different formula feeds, age of introduction of solid foods, type of weaning foods, whether any vitamins were taken and how much attention was paid to healthy eating (three point scale). Also included were questions about birth weight and whether the child had any other medical conditions.

4.3.6 Growth

Participants were weighed and measured as per standard clinic protocol during routine clinic visits. Infants under one year of age were weighed naked. Participants over one year of age were weighed clothed, but without shoes. Length was measured in children less than two years of age using a rollameter with the child lying supine. Height was measured using a stadiometer in children over two years of age. Head circumference was measured using standard clinic procedure. Weight was measured in kg to one decimal place. Height was measured in cm to one decimal place. Measurements were plotted on standard UK growth charts in the Personal Child Health Record (red book). Where it was not possible to weigh or measure the child on the day of completing the questionnaire, the parent was asked to have their child measured at the next health visitor clinic or asked to phone the researcher with the most recent measurements from their child's red book. Measurements were recorded if they were made within two months of the questionnaire completion date, as per the methodology of Wright et al. (2007).

4.4 Method

4.4.1 Study design

This was a cross sectional study of 8-30 month old children from the Isle of Wight. Figure 4.1 summarises the study design.

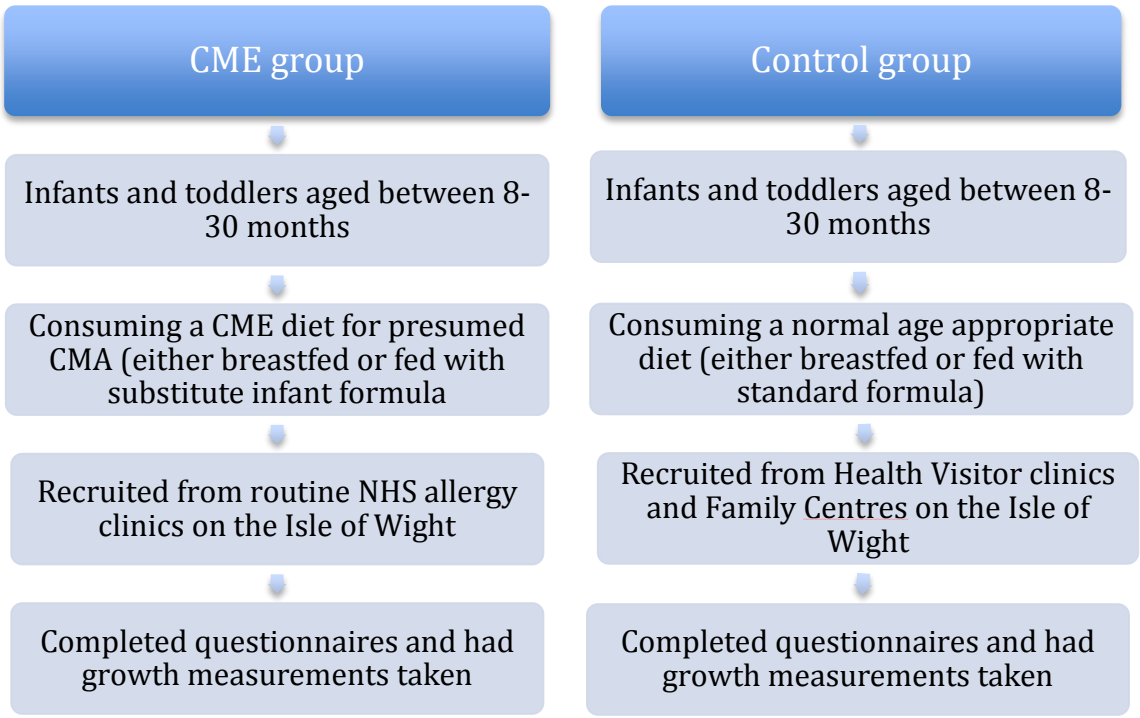

Figure 4.1 Summary of study design

4.4.1 Sample

4.4.1.1 Identification of participants

This study had two groups: an experimental group and a control group. The experimental group was composed of children consuming a CME diet for the management of documented or likely CMA. Children were eligible for the study if they had consumed a substitute formula and/or a CME diet in the first year of life for a period of three months or longer. Children who were excluding other foods (i.e. egg or soya), in addition to cows' milk were eligible to take part.

The control group was composed of children consuming an unrestricted diet. Children with any additional medical conditions requiring a special diet (e.g. Type 1 diabetes, cystic fibrosis) were excluded from the study.

4.4.1.2 Sample size

Sample size calculation was discussed and confirmed by the statistician at the University of Portsmouth School of Health Sciences and Social Work. All sample sizes were calculated using Gpower 3 for a two-tailed outcome, at 80% power and at a significance level of 0.05. Sample sizes were calculated for primary objectives only.

4.4.1.2.1 Fussy eating

Previous research on 12-month old infants using the Picky Eater questionnaire (Carruth et al., 1998) indicates mean scores of 2.5 (SD 0.8) on the pickiness subscale (Blossfeld et al., 2007). Assuming there will be a mean difference in score of 0.4 between groups requires 64 children in each group.

4.4.1.2.2 Food Neophobia

Previous research on 2-5 year old children indicates a mean score of 36.59 (SD 14) on the CFNS (Russell & Worsley, 2008). Assuming there will be a difference of one SD (14) between groups requires 16 children in each group.

4.4.1.2.3 Feeding difficulties

Previous research indicates a normative mean score of 31 on the Montreal Children's Hospital feeding scale for healthy children aged 6-24 months (personal communication with Dr. Maria Ramsay, February 2013). A score of 45 is diagnostic of feeding difficulties. Children with underlying medical diagnoses (e.g. gastrointestinal/neurological conditions) had a mean score of 60 (Ramsay et al., 2011). Assuming children with presumed or documented CMA will have a mean score of 40 (i.e. a higher score than normative, but not clinically diagnostic of feeding difficulties), 42 children are required in each group.

4.4.1.2.4 Dietary Variety (Food Variety Score)

No published studies have measured dietary variety in children under two years old using this method. A large-scale UK study of three year old children (n = 9796) (Emond et al., 2010) reported a mean Food Variety Score of 20.94 (SD 6.04). Assuming currently allergic children will have a 15% difference in Food Variety Score, requires 52 per group

In summary, to ensure the study is sufficiently powered for all of the primary objectives, requires a total of 132 children (64 in each group).

4.4.2 Ethical considerations

Ethical approval for the study was obtained from Berkshire NHS ethics committee in May 2013 (see appendix 15). Local approval was obtained from the Isle of Wight NHS Trust Research and Development Committee in July 2013 (see appendix 16). Parents were provided with a study information sheet and written informed consent was obtained (see appendices 18 and 19). Parents and children were free to withdraw from the study at any time. Data was anonymised and when not in use, secured in locked cabinets or password protected in the case

of electronic records. Online questionnaires were administered via Bristol online surveys (http://www.survey.bris.ac.uk), for which there is encryption, ensuring the data could not be intercepted by third parties.

4.4.3 Recruitment

Recruitment took place between July 2013 and December 2014. Children eligible for the experimental group were identified via NHS allergy clinics. A database of infants seen routinely in clinic for CMA was maintained by the researcher. All those who met the inclusion criteria for the experimental group were invited to participate in the study. It was originally planned to approach potential participants during their routine clinic appointment. However, in order to ensure that children were recruited in a timely manner (i.e. before cows' milk was reintroduced into their diet), parents were telephoned about the study approximately a month before their next planned clinic appointment. If parents expressed an interest in the study, an information sheet, consent form and questionnaire pack with prepaid return envelope were posted out. Questionnaires were returned by post or brought to the planned appointment. Questionnaires were also available to complete online. At the time of data collection, all those included in the experimental group had been seen by an allergy dietitian as part of their routine clinical care.

A control group of infants who met the inclusion criteria were recruited from identified health visitor clinics/family centres on the Isle of Wight. Following agreement with the clinic/centre manager, parents were approached and asked if they would like to participate in a study of infant and toddler eating habits. Parents who expressed an interest in participating in the study, were given a study information sheet and consent form, questionnaire pack and prepaid envelope. Parents were given the option to return the questionnaires by post or to complete them online.

4.4.4 Administration of questionnaires

Questionnaires were mostly self-administered, however the researcher was available to clarify any queries and double-check any omissions. For questionnaires completed at home and sent back by post, phone calls or emails were made to clarify any missing details. Medical notes were consulted to clarify any anomalies (e.g. timing of introduction of substitute formula).

4.4.5 Data analysis

4.4.5.1 Questionnaire coding

Questionnaires were scored and coded according to published guidelines by the original authors. Where the scoring of the questionnaires was not published, the author was contacted for this information. Mean values were calculated for the individual subscales of each questionnaire. A coding logbook was maintained to ensure consistency in coding of questionnaires. Questionnaire data was used if the respondent had completed at least 75% of questions. Ten per cent of files were double entered and compared to test consistency and minimise errors of data entry.

4.4.5.2 Statistical analyses

Data was analysed using SPSS software (IBM, version 20). Missing values were computed as discrete values. All data sets were double checked for outliers. Weight and height were converted into weight/age and height/age using the World Health Organisation (WHO) Anthro Plus software (WHO Reference 2007). Body Mass Index (BMI) was computed by dividing weight (kg) by height (m) squared and converted into z scores using the same software.

Diet Variety Score (DVS) was calculated as the number of times "never" is selected on the frequency option for each food. The DVS% for each category was calculated as a percentage of the items in each food category that had never been eaten (e.g. if 5 foods were scored as "never" consumed in a category of 10 foods, the DVS% is equal to 50% i.e. 50% of foods in that category are never eaten). Therefore a higher DVS% indicates a less varied diet is consumed.

All continuous variables were tested for normality of distribution using a one sample Kolmogorov-Smirnov test. Descriptive statistics were calculated for all variables. Categorical variables were expressed as numbers and frequencies. Continuous variables were expressed as mean and standard deviation (SD) or median and range, depending on normality of distribution.

As most of the variables were non-parametric, differences between the CME and control groups were compared using a Mann Whitney test for all primary outcome variables. For categorical variables, the X^2 test was used. Spearmann rho correlations were performed to identify any relation between the main outcome variables. A two way analysis of variance was used to measure the effect of one categorical variable, whilst controlling for another. Multiple regression calculations were performed to determine the contributing factors to the main outcome variables. The significance level was set at 0.05 for all analyses. The FFQ was

analysed using principal component analysis (PCA). PCA enables patterns of dietary intake to be explored rather than distinct dietary components, by grouping infants into clusters according to their intake and characteristics.

4.5 Results

4.5.1 Description of sample

In total 126 participants took part, 66 in the CME group and 60 in the control group. Demographic characteristics of participants are detailed in Table 4.1. There were slightly more males than females in the overall sample (67 and 59 respectively). Five infants were born preterm and 121 infants were born at term. Of those born preterm, the mean gestational age was 35.4 weeks. All participants were singleton births, except for one set of twins in the control group. 46 participants (36.5% of the sample) were 12 months or younger. The median age of the sample was 13.0 months. Participants in the CME group were younger than those in the control group ($p = 0.02$). Approximately one quarter of the total sample reported a history of maternal food allergy, with significantly higher rates in mothers of the CME group. There were no differences in gender, number of siblings, ethnicity, maternal age, parental occupational level or educational level between the two groups. The vast majority of questionnaires (96%) were completed by mothers.

Table 4.1 Demographic characteristics of all participants and by group

	All (*N* = 126)	CME group (*n* = 66)	Control group (*n* = 60)
Age (months) median	13.0 (8-27)	12.37* (8-25)	15.0* (8-27)
Female (%)	59 (46.8)	32 (48.5)	27 (45.0)
Male (%)	67 (53.2)	34 (51.5)	33 (55.0)
Maternal age (years) mean	29.3 (SD 6.5)	29.8 (SD 6.38)	28.6 (SD 6.62)
Number of siblings	1 (0-5)	0 (0-3)	0.5 (0-5)
Ethnicity			
White British (%)	118 (93.6)	61 (92.5)	57 (95.0)
Maternal occupation			
Student (%)	7 (5.6)	4 (6.1)	3 (5.0)
Self employed (%)	5 (4.0)	0 (0.0)	5 (8.3)
Full time (%)	21 (16.6)	11 (16.7)	10 (16.7)
Part time (%)	45 (35.7)	27 (40.9)	18 (30.0)
Unemployed (%)	21 (16.7)	13 (19.7)	8 (13.3)
Other (%)	27 (21.4)	11 (16.7)	16 (26.7)
Paternal occupation			
Student (%)	2 (1.6)	1 (1.5)	1 (1.7)
Self employed (%)	24 (19.0)	12 (18.2)	12 (20.0)
Full time (%)	78 (61.9)	39 (59.1)	39 (65.0)
Part time (%)	5 (4.0)	4 (6.1)	1 (1.7)
Unemployed (%)	9 (7.1)	6 (9.1)	3 (5.0)
Other (%)	2 (1.6)	2 (3.0)	0 (0.0)
Not stated	6 (4.8)	2 (3.0)	4 (6.6)
Maternal education			
None (%)	1 (0.8)	0 (0.0)	1 (1.7)
GCSE /A-level or equivalent (%)	80 (63.4)	41(62.1)	39 (65.0)
Graduate / Postgraduate (%)	41(32.6)	23 (34.9)	18 (30.0)
Not stated (%)	4 (3.2)	2 (3.0)	2 (3.3)
Paternal education			
None (%)	3 (2.4)	2 (3.0)	1 (1.7)
GCSE /A-level or equivalent (%)	82 (65.1)	43 (65.2)	39 (65.0)
Graduate / Postgraduate (%)	31(24.6)	15 (22.7)	16 (26.7)
Not stated (%)	10 (7.9)	6 (9.1)	4 (6.6)
Family history of food allergy			
Maternal (%)	32 (25.6)	24 (36.4)*	8 (13.3)*
Paternal (%)	12 (9.5)	9 (13.6)	3 (5.0)
Sibling (%)	18 (14.3)	14 (21.2)	4 (6.6)

*Difference between CME and control group significant < 0.05 using a Mann Whitney U test.
** Difference between CME and control group significant < 0.01 using a chi square test.

4.5.1.1 Anthropometric measurements

Details of birth weight, current weight, current length/height and current BMI are shown in Table 4.2. Current weight data was available for 108 participants and current length/height data was available for 55 participants. Participants in the CME group had a higher mean weight centile,

height centile and BMI centile than the control group, however this difference was not significant. For both groups these measurements were within the normal range. There was no significant difference between the CME and control group for any of the measurements. There was no significant difference for gender for any of the measurements.

Table 4.2 Anthropometric measurements of participants

	All (*N* = 126)	CME group (*n* = 66)	Control group (*n* = 60)
Birth weight (kg)	3.43 (1.55-4.67)	3.48 (2.08 – 4.67)	3.34 (1.55 – 4.53)
Weight (kg)	9.9 (7.43-14.90)	9.9 (7.59-14.9)	10.1 (7.43 – 14.9)
Length / height (cm)	76.0 (68-90.4)	76.0 (69.0 -90.4)	76.0 (68.0-88.0)
Weight centile (%)	62.2 (5.6-137.0)	65.9 (5.6-137.0)	52.2 (8.1 – 98.2)
Weight/age z score (SD units)	0.31 (1.01)	0.41 (1.09)	0.18 (0.89)
Length/height centile (%)	66.9 (3.9-110.0)	67.8 (3.9 -100)	30.8 (11-110)
Height/age z score (SD units)	0.44 (-1.76-3.91)	0.46 (-1.76-3.91)	0.5 (-1.19-3.41)
BMI ($kg/m^{2)}$	17.0 (14-20.6)	17.1 (14-20.6)	17.0 (14.3-19.0)
BMI centile (%)	65.3 (2.6-99.5)	67.4 (2.6 – 99.5)	54.9 (8.2 – 97.1)
BMI Z score (SD units)	0.39 (-1.95-2.58)	0.45 (-1.95-2.58)	0.12 (-1.39-1.9)

Median values shown. Minimum and maximum values in brackets.

4.5.1.2 Infant feeding characteristics of sample

Details of participants' breastfeeding status, use of formula milk and weaning foods are shown in Table 4.3. The majority of infants had been breastfed at some stage (81%), but only 13.5% were being breastfed at the time of data collection. There was no difference in the number of months of breastfeeding between groups, however significantly more of the CME group (97.0%) than the control group (82.3%) had ever been given formula milk ($p = < 0.01$). An older age at introduction of formula milk was significantly correlated with maternal education level ($rho = 0.305$, $p < 0.01$) and maternal age ($rho = 0.218$, $p = 0.021$)

The median age at solid food introduction was 20 weeks. Baby rice was the most common first solid food for both groups. Approximately half of the overall sample was fed predominantly with homemade weaning food (50.8%). Infants in the control group were commenced on solid food ($p = 0.033$), lumpy food ($p = 0.049$) and finger foods ($p < 0.01$) significantly earlier than the CME group.

Table 4.3 Infant feeding characteristics of participants

	All (*N* = 126)	CME group (*n* = 66)	Control group (*n* = 60)
Currently being breastfed (n, %)			
Yes	18 (14.3)	8 (12.1)	10 (16.7)
No	108 (85.7)	58 (87.9)	50 (82.3)
Ever been breastfed (n, %)			
Yes	102 (81.0)	54 (81.8)	48 (80.0)
No	24 (19.0)	12 (18.2)	12 (20.0)
Ever given formula milk (n, %)			
Yes	115 (91.3)	64 (97.0)*	50 (82.3)*
No	11 (8.7)	2 (3.0)	10 (16.7)
Breastfeeding duration (n, %)			
Never	24 (19.0)	12 (18.2)	12 (20.0)
< 1 month	31 (24.6)	17 (25.7)	14 (23.3)
1-3 months	16 (12.7)	11 (16.6)	5 (8.3)
3-6 months	20 (15.9)	12 (18.2)	8 (13.3)
6-9 months	12 (9.5)	6 (9.1)	6 (10.0)
9-12 months	6 (4.8)	4 (6.1)	2 (3.3)
>12 months	17 (13.5)	4 (6.1)	13 (21.7)
First weaning food (n, %)			
Baby rice	51 (40.5)	24 (36.4)	27 (45.0)
Fruit	39 (30.9)	20 (30.3)	19 (31.7)
Sweet potato/carrot/parsnip	24 (19.0)	13 (19.7)	11 (18.3)
Broccoli	2 (1.6)	1 (1.5)	1 (1.7)
Rusk/biscuit	4 (3.2)	4 (6.1)	0 (0.0)
Porridge	2 (1.6)	2 (3.0)	0 (0.0)
Other	4 (3.2)	2 (3.0)	2 (3.3)
Age solid food introduction (weeks) (*n, %)*	20 (5.5-30)**	20.0 (5.5-30)**	18 (12-28)**
Age lumpy food introduction (weeks) (*n, %)*	26 (12-42)**	26.5 (12-42)**	25 (16-36)**
Age finger food introduction (weeks) *(n, %)*	26 (16-52)^	26.5 (20-52)^	24 (16-36)^
Type of weaning food (n, %)			
Homemade	64 (50.8)	33 (50.0)	31 (51.7)
Prepared baby food	9 (7.1)	7 (10.6)	2 (3.3)
A mixture of both	53 (42.1)	26 (39.4)	27 (45.0)
Dietary supplement (n, %)			
Yes	27 (21.4)	12 (18.2)	15 (25.0)
No	99 (78.6)	54 (81.8)	45 (75.0)

*Difference between CME and control group significant < 0.01 using a chi square test
** Difference between CME and control group significant < 0.05 using a Mann Whitney test.
^ Difference between CME and control group significant < 0.01 using a Mann Whitney test.

4.5.1.3 Dietary exclusion

By definition, all of the CME group was excluding cows' milk from their diet and none of the control was excluding any foods from their diets. The number and type of foods excluded by

the CME group is displayed in Figure 4.2. The majority (71.2%) of the CME group was excluding cows' milk only, whilst 28.8% were excluding another foods in addition to cows' milk. The most number of foods excluded was three. Cows' milk was excluded by the CME group at a median age of 9.5 weeks (range 1-30), which includes the exclusion of cows' milk from maternal diet in those breastfeeding. Participants in the CME group had a median 3.0 (range 1-18) contacts with an allergy dietitian, which included both telephone and face-to-face contacts. None of the participants in the control group had ever seen a dietitian for any reason.

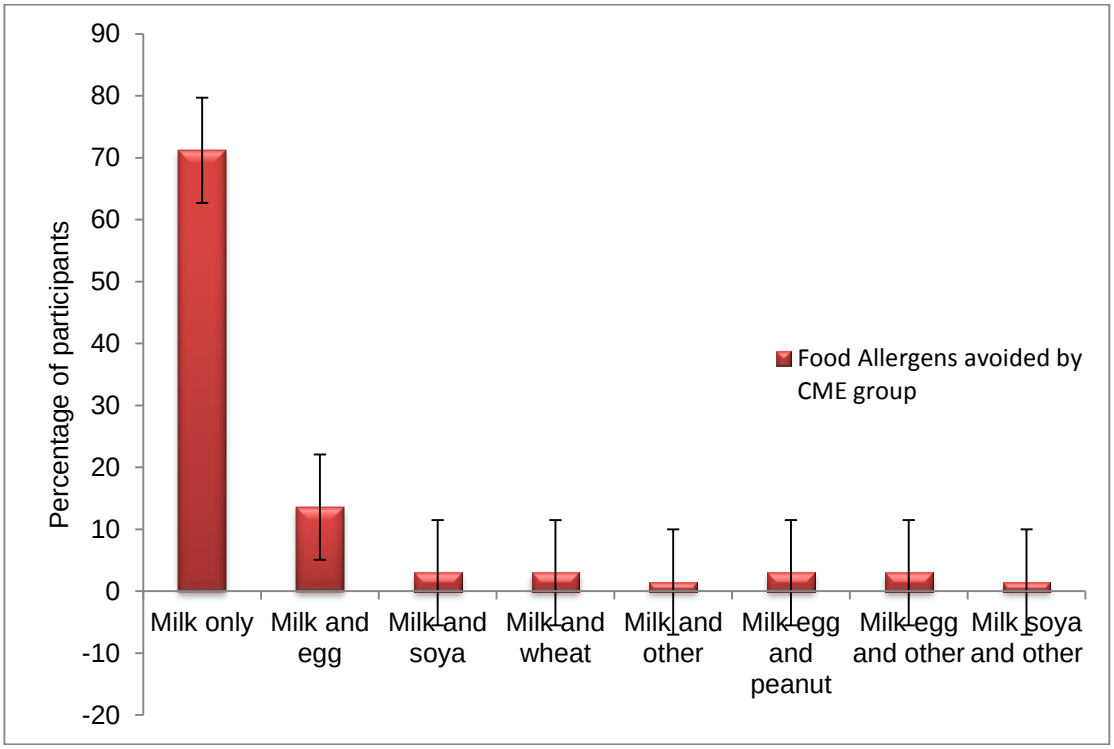

Figure 4.2 Foods excluded by the CME group

4.5.1.4 Use of substitute formula

Of the 66 infants in the CME group, three were breastfed as their main source of milk and did not have any substitute formula. All three mothers were consuming either a partial or complete CME diet. In the rest of the group (n = 63), a substitute formula for CMA was commenced at a median age of 11.0 weeks (range 1-37 weeks). At the time of data collection, the median duration of usage of a substitute formula was 41.0 weeks (range 2-91 weeks).

The type of substitute formula being used at the time of data collection is shown in Figure 4.3. Substitute formula were initiated by a number of different health professionals: GPs, health visitors, two allergy dietitians and paediatricians with a special interest in allergy. The

most commonly used substitute formula was an Amino Acid Formula (AAF) (Neocate LCP) (n = 30, 45.5%). Of the 30 participants being fed an AAF, 18 (60%) had only had this specific substitute formula, whereas 12 (40%) had trialed a different substitute formula before Neocate LCP. One infant had trialed a soya formula, one had trialed a partially hydrolysed formula (a commercially available “comfort” milk), four had trialed a whey EHF and six had trialed a casein EHF. The median duration of trialing a different substitute formula before progressing to an AAF was 5.0 weeks (range 2-16 weeks).

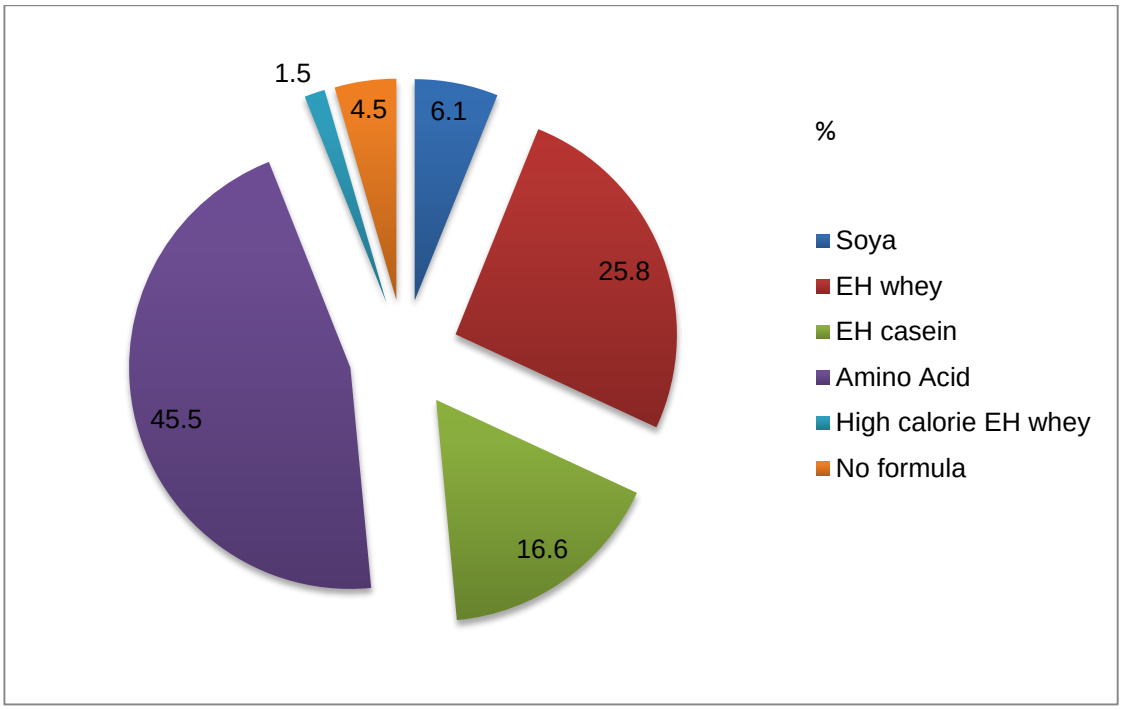

Figure 4.3 Type of substitute formula used by CME group

4.5.1.5 Reported symptoms

The median number of symptoms reported by all participants was 3.0 (range 0-7). There was no difference in the number or type of symptoms reported by gender and no correlation between number of symptoms and age of participant or maternal age. Participants in the CME group reported a median number of 4.0 symptoms (range 1-7), which was significantly higher than that reported by the control group (median 2, range 0-6) ($p < 0.01$). Participants whose mother had a history of food allergy symptoms had significantly more symptoms reported ($p < 0.01$), with reported higher rates of vomiting (chi square $p = 0.037$), abdominal pain (chi square $p = 0.01$) and colic (chi square $p < 0.01$) than those with no maternal family history of food

allergy symptoms. Paternal or sibling food allergy symptoms had no effect on the number or type of symptoms reported.

The types of symptoms reported by respondents are shown in Figure 4.4. Overall the most commonly reported symptoms for all participants were vomiting (57.1%) and colic (50.0%). The least commonly reported symptom for all participants was abdominal distension (15.1%). There were significantly higher rates of vomiting (chi square p = 0.014), constipation (chi square p = 0.014), abdominal distension (chi square p < 0.01), colic (chi square p < 0.01) and "other food-related problems" (chi square p < 0.01) reported in the CME group than the control group. There was no difference in the rates of wheezing/whistling in the chest (chi square p = 0.572), dry cough at night (chi square p = 0.531), eczema (chi square p = 0.125) or diarrhea (chi square p = 0.775) between groups.

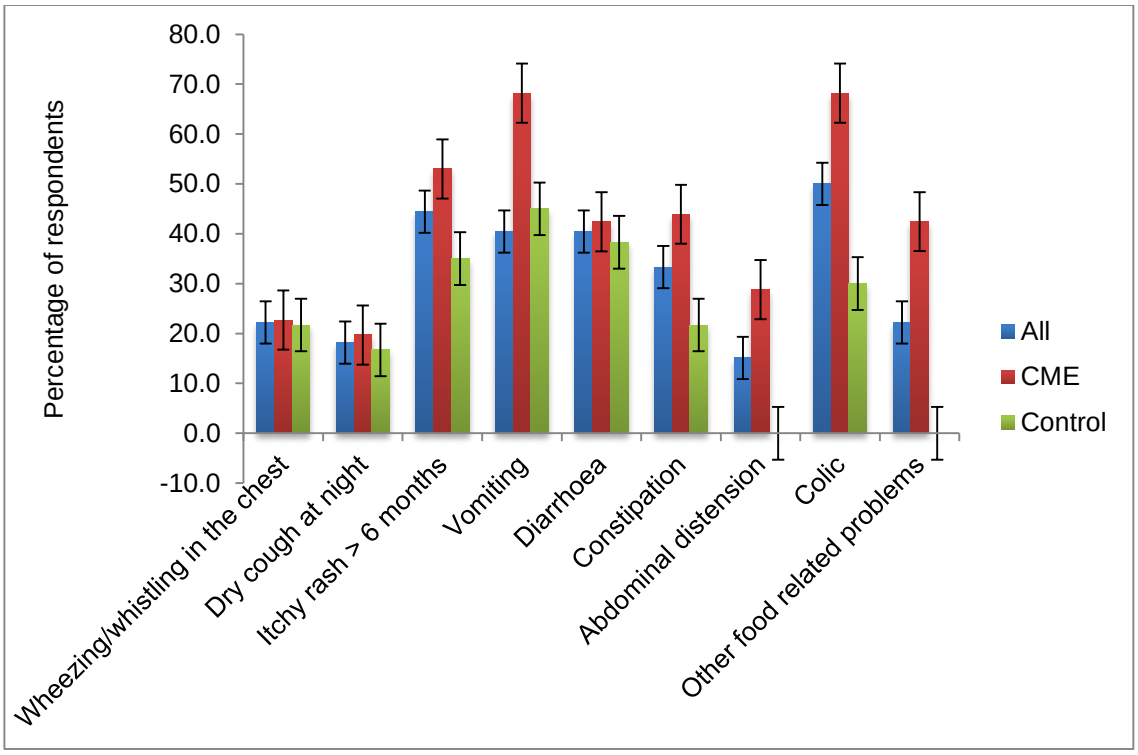

Figure 4.4 Symptoms reported by respondents

"Other food related problems" were reported by 28 (42.4%) of the CME group. The most common was urticaria, reported by 11 respondents and blood in stools, reported by six. The other symptoms reported are shown in Figure 4.5.

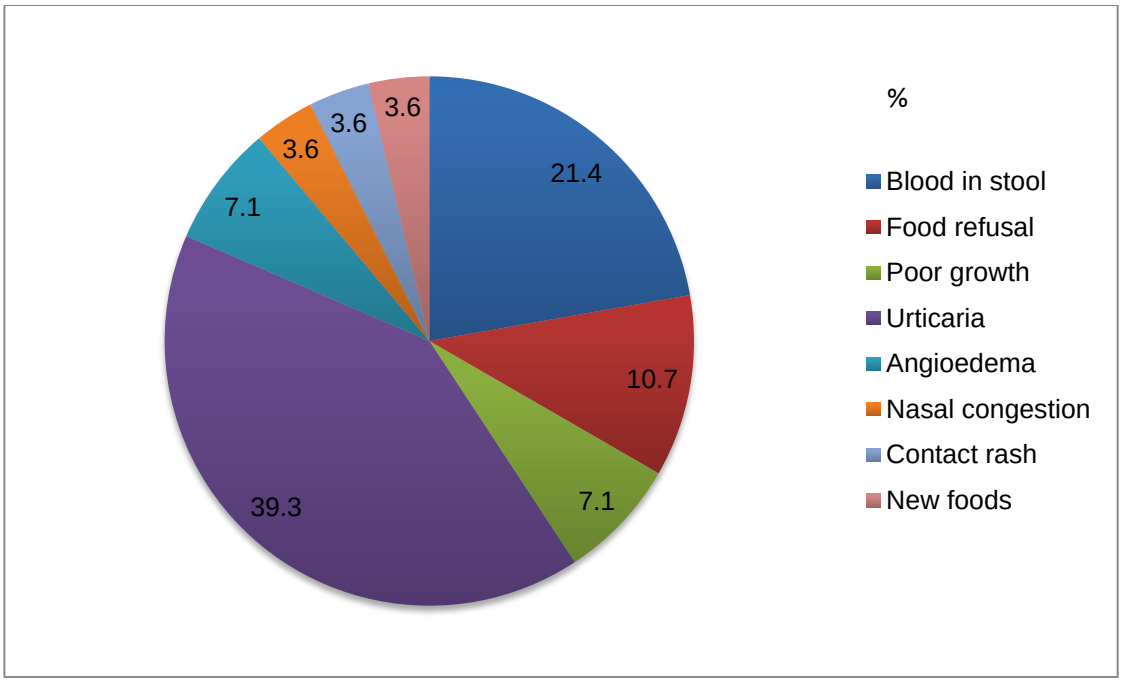

Figure 4.5 Other "food-related problems" reported by the CME group

4.5.1.6 Skin Prick Test (SPT) status

All participants in the CME group had a SPT to cows' milk reagent as part of routine clinical practice, with twenty participants (30.3%) having a positive SPT (> 3mm). Differences between those with a positive SPT and negative SPT are shown in Table 4.4. Participants who had a positive SPT to cows' milk reported more symptoms of eczema (chi square $p = 0.039$) and "other food related problems" (chi square $p < 0.01$) than those with a negative SPT. Participants with a negative SPT reported more symptoms of constipation (chi square $p < 0.01$), abdominal distension (chi square $p < 0.01$) and colic (chi square $p < 0.01$). There was no difference in levels of wheeze, cough or diarrhea by SPT status. Those with a positive SPT also had significantly more symptoms ($p < 0.01$).

Participants with a positive SPT avoided a higher number of foods than those with a negative SPT ($p < 0.01$) and their weight/age centile was significantly lower. To investigate whether weight/age centile was related to the number of foods avoided in the CME group as a whole, a two-way ANOVA was conducted to explore this link. Participants were divided into three groups based on the number of foods being excluded (one, two or three). There was no significant difference in weight/age centile according to number of foods excluded ($p = 0.947$) between groups or within groups. This was conducted on the whole CME group, rather than just the SPT positive subset of the group, in order to increase the power of the calculation.

Table 4.4 Comparison of participants in the CME group with positive and negative SPT

	SPT positive (*n* = 20)	SPT negative (*n* = 46)	p value
Number of symptoms reported	2 (1-7)	4 (1-7)	0.006**
Number of foods excluded	1.5 (1-3)	1 (1-3)	0.031*
Age of introduction of substitute formula	20 (3-37)	8 (1-28)	0.013*
Duration of substitute formula (weeks)	28.5 (2-91)	42 (18-89)	0.030*
Age of introduction of solids (weeks)	20 (16-26)	20 (5.5-30)	0.174
Weight/age centile (%)	45.3 (5.6-98.8)	70.4 (6.4-137)	0.028*
Height/age centile (%)	75.1 (5-97.3)	67.6 (3.9-100)	0.517
BMI centile (%)	47.2 (11.3-98.8)	67.8 (2.6-99.5)	0.486

Median number shown with minimum-maximum values in brackets. *p < 0.05

4.5.2 Main outcome measures

4.5.2.1 Feeding difficulties

Data was available for all 126 participants. The overall median feeding difficulty score was 24.5 (range 15-68). The minimum and maximum scores possible on this questionnaire are 14 and 98 respectively. The median score in the CME group (26.5, range 16-68) was significantly higher than that of the control group (22.0, range 15-53) ($p < 0.01$), indicating higher levels of feeding difficulties, although both groups were within the normal range. Nine participants in the CME group (13.6%) had scores diagnostic of clinical feeding difficulties (score > 45), compared to only one participant in the control group (1.6%). There was no effect of gender, being older or younger than one year, or breastfeeding status on feeding difficulty score. Across the group as a whole, participants whose mothers had a history of food allergy symptoms recorded significantly higher scores of feeding difficulties ($p = 0.03$).

Looking at the whole sample, a number of factors was found to be significantly positively correlated with higher feeding difficulty scores using a Spearman Rank Order correlation (rho). The number of reported allergic symptoms was the most strongly correlated factor, demonstrating a moderate correlation of rho = 0.352 ($p < 0.01$). A number of individual symptoms were also found to be weakly correlated with a higher feeding difficulty score, specifically: colic (rho = 0.281, p = 0.01), wheeze (rho = 0.197, p =-0.027), dry cough rho = 0.271, $p < 0.01$), vomiting (rho = 0.213, p = 0.017) constipation (rho = 0.213, p = 0.017) and “other food related problems” (rho = 0.226, p = 0.01). The volume of milk/milk substitute

consumed per day was also weakly correlated with feeding difficulty score (rho = 0.218, p = 0.04). Overall there was no correlation found between feeding difficulty score and age of participant, gender, maternal age, parental education/occupation, birth weight, current weight for age, number of siblings, symptoms of itchy rash, diarrhea or abdominal distension, age of introduction of solid food, lumpy food or finger foods, type of first weaning food, breastfeeding status or duration.

Within the CME group, the total number of symptoms and some specific symptoms were found to be significantly correlated with a higher feeding difficulty score. These are listed in Table 4.5. In addition, the volume of milk substitute formula consumed per day, "attention paid to healthy eating" and number of dietetic contacts were also correlated with a higher feeding difficulty score. Age at time of dietary exclusion was inversely correlated with feeding difficulty score. Age, gender, maternal age, parental education/occupation, birth weight or current weight for age, number of siblings, duration of breastfeeding, age of introduction of solid/lumpy food, SPT status, number of foods excluded, duration and type of substitute formula and duration of exclusion diet were not correlated with feeding difficulty score.

Table 4.5 Factors correlated with higher feeding difficulty scores in the CME group

	Correlation coefficient (rho)	p value
Number of symptoms reported**	0.355	0.003
Number of contacts with dietitian*	0.263	0.033
Symptom of colic*	0.252	0.041
Symptom of wheeze**	0.357	0.003
Symptom of dry cough at night**	0.358	0.003
Volume of milk substitute per day**	0.327	0.007
Age at time of dietary exclusion*	-0.249	0.044
Attention paid to healthy eating*	0.251	0.042

* $p < 0.01$ **$p < 0.05$

Multiple regression analyses was undertaken on the CME group to determine the ability of several factors to predict the level of feeding difficulties using a standard multiple regression entry process. There was no violation of the assumptions of normality, linearity, multicollinearity and homoscedasticity. In the final model, 41.3% of the variance in feeding

difficulties could be explained (R = 0.642, SE 11.09). A history of colic made the most contribution to this model (β score = -0.459, p = 0.03). Three variables made a unique statistically significant contribution (colic, dry cough at night and other food related problems).Details are shown in Table 4.6.

Table 4.6 Multiple regression model explaining 41.3% of variance in feeding difficulty score in the CME group

	B	*SE B*	*β*	*t*	*p*
Number of symptoms reported	-2.814	1.840	-.351	-1.529	.132
Lumpy food introduction age	.460	.254	.195	1.810	.076
Wheezing	-9.035	4.504	-.285	-2.006	.050
Dry cough at night	-14.991	4.539	-.448	-3.303	.002
Maternal food allergy	3.249	3.186	.124	1.020	.312
Vomiting	-5.675	3.823	-.199	-1.484	.144
Constipation	-3.349	3.508	-.125	-.955	.344
Colic	-11.390	3.622	-.459	-3.145	.003
Other food related problems	-7.630	3.133	-.286	-2.435	.018

4.5.2.2 Fussy Eating

Data was available for all 126 participants. The median score for all participants was 21. The minimum and maximum possible scores for this questionnaire are 10 and 70 respectively. Overall there was no difference in scores for gender, being older or younger than one year, maternal food allergy history or breastfeeding status. The CME group had a significantly higher median score (22.5, range 10-63) than the control group (18.0, range 10-44) ($p < 0.01$), indicating they have higher levels of fussy eating, although both groups' median scores were within the normal range.

Looking at all participants, there was no correlation between fussy eating score and maternal age, parental education/occupation, number of siblings, family food allergy history, gestational age, birth weight or current anthropometric status or age of any type of solid food introduction. There was also no correlation found for symptoms of eczema, abdominal distention, constipation, vomiting or diarrhea. The factors that were significantly positively correlated with fussy eating scores using a two-tailed Spearmann correlation were number of allergic symptoms (rho = 0.281, $p < 0.01$), colic (rho = 0.212, p = 0.017), wheeze (rho = 0.204, p = 0.02) and dry cough (rho = 0.225, p = 0.01).

Within the CME group, there was no correlation between fussy eating score and age at

introduction of substitute formula, duration of substitute formula consumption, type of substitute formula or SPT status. There was no correlation between any specific symptom or any demographic or infant feeding factors. A positive correlation was found for volume of milk substitute consumed per day (rho = 0.305, p = 0.013).

Multiple regression analyses was undertaken on the CME group to determine the ability of several factors to predict the level of fussy eating using a standard multiple regression entry process. There was no violation of the assumptions of normality, linearity, multicollinearity and homoscedasticity. In the final model, 21.4% of the variance in fussy eating could be explained (R = 0.462, SE 11.22). The total volume of milk/milk substitute consumed/day made the most contribution to this model (β score = -0.258, p = 0.039) and was the only variable to make a unique statistically significant contribution. Details are shown in Table 4.7.

Table 4.7 Multiple regression model explaining 21.4% of the variance in fussy eating scores in the CME group

	B	*SE*	*β*	*t*	*p*
Number of symptoms reported	-.871	1.372	-.122	-.635	.528
Colic	-4.450	3.514	-.201	-1.266	.210
Age of child	.703	.354	.257	1.987	.052
Number of foods excluded	-2.934	2.426	-.154	-1.209	.231
Wheeze	-6.233	4.461	-.220	-1.397	.168
Dry cough	-3.290	4.218	-.110	-.780	.439
Volume milk substitute/day	.012	.006	.258	2.108	.039

4.5.2.3 Food neophobia questionnaire

Data was available for 117 participants (92.9%). Nine respondents (7.1%) did not complete all or part of the questionnaire. Seven did not complete the questionnaire as the questions were felt not to be relevant to their child due to their young age (mean age of the subgroup of participants was 9.5 months). Two did not complete the questionnaire due to lack of time.

The median score for all participants was 21. The minimum and maximum possible scores for this questionnaire are 10 and 70 respectively, with a higher score indicating higher levels of food neophobia. Overall there was no difference in scores for gender, being older or younger than one year, breastfeeding status or maternal history of allergy. The CME group had a significantly higher median score (22.3 range 10-65) than the control group (19, range 10-51) ($p < 0.05$), indicating they have higher levels of food neophobia.

As was also the case for feeding difficulties and fussy eating, across the whole group of participants, there was no correlation between food neophobia score and maternal age, parental educational or occupation level, number of siblings, family food allergy history, birth weight or current anthropometric status, breastfeeding duration or age of any type of solid food introduction. The factors that were significantly correlated with food neophobia scores using a two-tailed Spearmann rho correlation are shown in Table 4.8. The factor that was most strongly correlated with food neophobia score was the number of contacts with dietitian (rho = 0.272, $p < 0.01$). Within the CME group, there was no correlation between food neophobia score and age at introduction of substitute formula or duration of substitute formula consumption or SPT status.

Table 4.8 Factors correlated with higher food neophobia scores

	Correlation coefficient (rho)	p value
Number of reported symptoms*	0.193	0.037
Number of contacts with dietitian**	0.272	0.003
Number of foods excluded*	0.200	0.031

* $p < 0.05$ **$p < 0.01$

4.5.2.4 Food frequency questionnaire

Data was available for all 126 participants. The median volume of cow's milk/cows' milk substitute consumed by participants per day was 480mls (range 0-1080mls). This intake was higher in the CME group (median 487mls), but the difference was not significant. Higher volumes of milk/milk substitute were consumed by participants under one year old ($p < 0.01$) and lower volumes were consumed by those being breastfed ($p < 0.01$).

Differences in the frequency of consumption of individual foods between the CME group and control group are detailed in Table 4.9. Foods are categorised into three different groups depending on whether the control group or CME group consumed them more or less frequently. For the majority of individual foods (59.5%), there was no significant difference in consumption between groups.

Table 4.9 Differences in frequency of food consumption between groups

Category	CME group consumed more frequently than control group*	CME group consumed less frequently than control group*
Beverages	None	Baby juice Tea
Readymade baby food	Dried meat/fish dish Dried vegetable/pasta/rice dish Dried dessert Readymade breakfast meals Readymade meat/fish meal, Readymade vegetable/pasta/rice meal Readymade fruit puree Readymade fruit dessert	None
Starchy carbohydrates	None	Breakfast cereal/porridge
Dairy, egg and soya substitutes	Soya cheese Soya yogurt Other milk substitute yoghurt	Cheese Savoury white sauce Yoghurt/fromage frais Pizza Quiche Eggs
Meat and fish	None	Meat pie sausage roll Ham/processed cold meats Oily fish Tuna
Fruit	None	Banana Orange/Satsuma
Vegetables	Carrot	None
Sweet and miscellaneous foods	None	Ice cream Custard Cakes/buns/pastries Chocolate/digestive biscuit Chocolate Sweets Butter/Margarine

*$p < 0.05$

Differences in consumption of different food categories between the CME and control groups are shown in Table 4.10. The food categories with the biggest differences between the CME and control groups were “readymade baby food”, “dairy/egg”, “soya/substitute”, “non-water drinks” and “sweet /miscellaneous” foods.

Table 4.10 Frequency of consumption of different food categories per week

	CME group (*n* = 66)	Control group (*n* = 60)	p value
Non water drinks	0.5 (0.0-32)	3.5 (0.0-35)	0.013*
Readymade baby food	7.5 (0.0-60)	0.5 (0.0-23)	0.000*
Cereals	24.0 (10.5-42.5)	22.5 (0.5-44)	0.689
Dairy products/Eggs	0.0 (0.0-3)	15.0 (3.0-30)	0.000**
Soya products	3.0 (0.0-18)	0.0 (0-0.5)	0.000**
Meat and fish	7.5 (0.0-23.5)	9.5 (0.5-33)	0.138
Vegetables	21.5 (1.5-59.0)	19.75 (6-47)	0.470
Fruit	18.0 (3.5-50.0)	20.75 (1.0-55)	0.232
Sweet/Miscellaneous	10.0 (0.0-33.0)	16.0 (0.5-51)	0.000**
All foods	96.3 (61.5-218.5)	102.5 (53.5 – 218.5)	0.024*

Median values shown (minimum-maximum). * Statistically significant Mann Whitney Test < 0.05. **Statistically significant Mann Whitney Test < 0.01

There was no difference between genders for frequency of consumption of individual foods. Significant differences in consumption of foods were found between participants under and over one year old. To investigate this further, the group was stratified according to age (under one year and over one year). Separate Mann Whitney U tests were performed between the CME and control group for those participants under and over one year. Differences between consumption of dairy/egg products, soya/substitute products, readymade baby foods, non-water drinks and sweet/miscellaneous food groups were calculated. No difference was found in the consumption of readymade baby food between the two groups in participants under one year of age ($p = 0.460$), however in children over the age of one, those in the CME group consumed readymade baby food significantly more frequently compared with those in the control group ($p < 0.01$). Similarly, in terms of sweet/miscellaneous foods, there was no difference in consumption between the two groups of infant under one year of age ($p = 0.094$), however over one year of age, those in the control group consumed significantly more than those in the CME group ($p < 0.01$). Differences in consumption of dairy/egg products and soya/substitute foods persisted between groups across both age groups ($p < 0.01$ for at both ages). Differences in consumption of non-water drinks was approaching significance in participants under one year of age ($p = 0.05$), but was not significant in participants over one

year of age (p = 0.312)

4.5.2.5 Diet Variety Score (DVS) and Diet Variety Score % (DVS%)

Diet Variety Score was calculated as the number of times “never” is selected on the frequency option for each food. From the DVS, the DVS% for each category was calculated as a percentage of the items in each food category that had never been eaten. Therefore a *higher* DVS and DVS% indicate a *less* varied diet is consumed.

The overall DVS for all foods was significantly higher in the CME group ($p < 0.01$) meaning those excluding cows milk from their diet have a less varied diet overall than those consuming a normal diet. This calculation was repeated for all foods without the dairy/egg/soya substitute category, to control for reverse causality and the same difference was found with the control group having a more varied diet ($p < 0.01$). Looking at individual food categories, the median DVS% for readymade baby food was significantly lower in the CME group than the control group ($p < 0.01$) (i.e. the CME group consume a greater variety of readymade baby food than those in the control group). The DVS% in the dairy ($p < 0.01$), meat ($p < 0.01$) and sweet/miscellaneous ($p < 0.01$) food groups were significantly higher in the CME group than the control group (i.e. the control group consume a greater variety of these food groups than the CME group). This is displayed in Figure 4.6.

There was no difference in DVS% for any food group according to gender. There were some significant differences found according to age. Participants who were over one year old had significantly lower DVS% scores for all food groups than those who were under one year old ($p < 0.01$), meaning older children ate a more varied intake of all individual foods groups. In terms of breastfeeding status, infants who were currently being breastfed were fed a wider variety of fruit ($p = 0.018$) and a reduced variety of drinks ($p = 0.04$). As expected a correlation was found between number of foods excluded and increased DVS for total foods ($rho = 0.385$, $p < 0.01$).

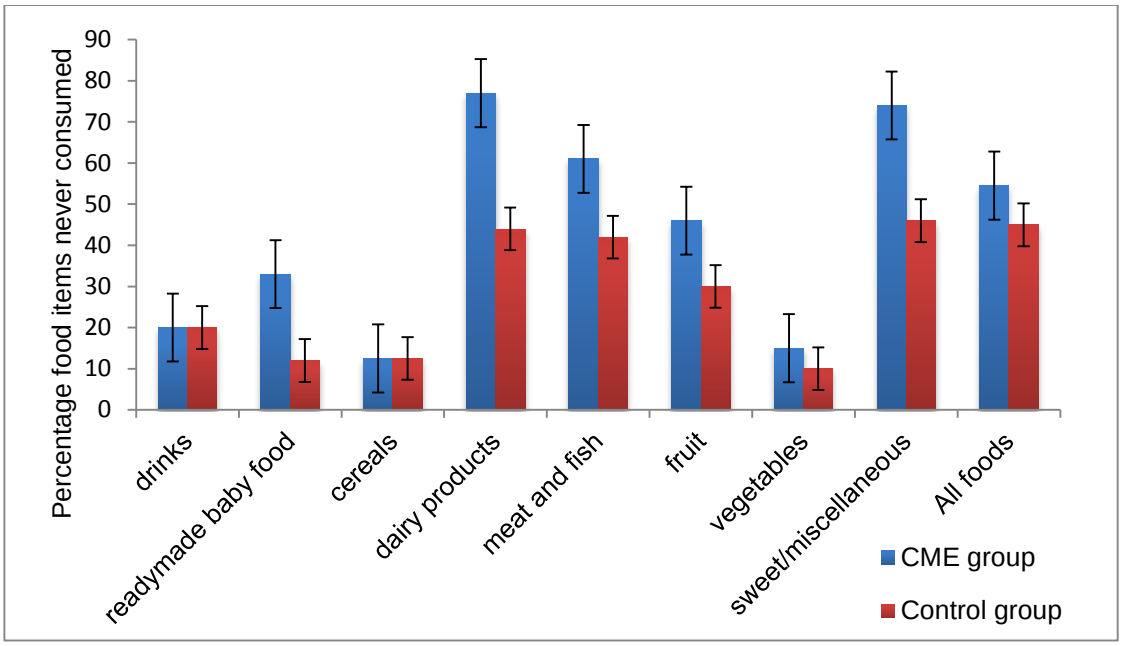

Figure 4.6 Diet variety score for each food category by group

Multiple regression analyses was undertaken to determine the ability of several factors to predict the DVS for all foods using a standard multiple regression analysis entry process. There was no violation of the assumptions of normality, linearity, multicollinearity and homoscedasticity. In the final model, 35.3% of the variance in dietary variety could be explained (R = 0.594, SE 7.67). Four factors contributed to the model: age, fussy eating, feeding difficulty and number of foods excluded. Details are shown in Table 4.11.

Table 4.11 Multiple regression analysis model explaining 35.3% of variation in DVS

	B	***SE B***	***β***	***t***	***p***
Age	-.764	.141	-.422	-5.425	.000
Number of foods excluded	3.166	.858	.281	3.689	.000
Feeding difficulty score	-.199	.111	-.246	-1.794	.075
Fussy eating score	.454	.123	.511	3.703	.000

4.5.2.6 Correlation of main outcome variables to each other

The multiple regression analysis model above indicates that both fussy eating and feeding difficulties are predictive of the overall dietary variety of the diet in this group of participants. This illustrates that there are significant correlations between the main outcome variables, detailed in Table 4.12. As can be seen, the variables that are most highly correlated are feeding difficulties and fussy eating (rho= 0.830, $p < 0.01$).

Table 4.12 Correlation of main outcome variables to eachother

		DVS all foods	Feeding difficulties	Fussy eating	Food neophobia
DVS all foods	rho	1.000	.202*	.236**	.227*
	p	.	.023	.008	.014
Feeding	rho	.202*	1.000	.830**	.625**
difficulties	p	.023	.	.000	.000
Fussy eating	rho	.236**	.762**	1.000	.701**
	p	.008	.000	.	.000
Food neophobia	rho	.227*	.625**	.701**	1.000
	p	.014	.000	.000	.

*Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).

To examine these relationships further, Spearmann rho correlations were calculated for the three main outcome variables and consumption of each food category. To gain a better understanding of dietary *patterns* of the group of participants as a whole, irrespective of dietary exclusion status, correlations were also calculated between consumption of different food categories. Details are shown in Table 4.14.

There were weak inverse correlations found between both fussy eating score, food neophobia and vegetable intake (rho = -0.196 and rho -0.201 respectively). Fussy eating and food neophobia were correlated with DVS for all foods (rho = 0.293 and rho = 0.280 respectively). Likewise, feeding difficulty score was weakly inversely correlated with fruit consumption (rho = -0.189) and positively correlated with DVS for all foods (rho = 0.255). All three main outcome variables were inversely correlated with dairy/egg consumption and positively correlated with soya/substitute intake consumption. None of the three outcome variables were correlated with consumption of meat/fish, sweet/miscellaneous foods or cereal categories. Some of the other food categories were correlated to each other; namely fruit and vegetables (rho = 0.533) non-water drinks and sweet/miscellaneous foods (rho = 0.472), cereals and meat/fish (rho = 0.388).

Table 4.13 Correlation of outcome variables with consumption of food categories. *significant p < 0.05 **significant p < 0.01

	Fussy eating	Feed Diff.	Neophobia	Sweet/ Misc.	Dairy/ Egg	Soya	Fruit	Veg.	Meat/ Fish	Readymade baby food	Cereals	Non water drinks	DVS all
Fussy eating	1	**.830****	**.795****	.027	**-.203***	**.204***	-.168	**-.196***	-.026	.078	.020	.062	**.293****
		.000	.000	.764	.023	.022	.059	.028	.775	.386	.825	.488	.001
Feeding difficulty	**.830****	1	**.675****	-.005	**-.251****	**.187***	**-.180***	-.160	-.082	.096	-.032	.045	**.255****
	.000		.000	.960	.005	.036	.044	.074	.363	.284	.725	.614	.004
Sweet/ Misc.	.027	-.005	.041	1	**.465****	-.137	**.225***	.079	**.299****	**-.249****	**.291****	**.472****	**-.445****
	.764	.960	.660		.000	.125	.011	.382	.001	.005	.001	.000	.000
Neophobia	**.795****	**.675****	1	-.041	**.244****	**.185***	.137	**-.210***	.101	-.081	-.084	-.151	**.280****
	.000	.000		.660	.008	.046	.140	.023	.278	.385	.366	.103	.002
Dairy/Egg	**-.203***	**-.251****	**.244****	**.465****	1	**-.470****	**.272****	.112	**.290****	**-.427****	.123	**.276****	**-.496****
	.023	.005	.008	.000		.000	.002	.213	.001	.000	.171	.002	.000
Soya	**.204***	**.187***	**.185***	-.137	**-.470****	1	-.009	.051	.071	**.216***	.127	-.103	.085
	.022	.036	.046	.125	.000		.920	.573	.431	.015	.155	.253	.343
Fruit	-.168	**-.180***	.137	**.225***	**.272****	-.009	1	**.533****	**.209***	-.056	**.272****	**.191***	**-.423****
	.059	.044	.140	.011	.002	.920		.000	.019	.535	.002	.032	.000
Vegetables	**-.196***	-.160	**-.210***	.079	.112	.051	**.533****	1	**.269****	-.053	**.273****	.060	**-.216***
	.028	.074	.023	.382	.213	.573	.000		.002	.559	.002	.505	.015
Meat/Fish	-.026	-.082	.101	**.299****	**.290****	.071	**.209***	**.269****	1	**-.338****	**.368****	**.249****	**-.387****
	.775	.363	.278	.001	.001	.431	.019	.002		.000	.000	.005	.000
Readymade baby food	.078	.096	-.081	**-.249****	**-.427****	**.216***	-.056	-.053	**-.338****	1	-.089	-.122	**.231****
	.386	.284	.385	.005	.000	.015	.535	.559	.000		.323	.173	.009
Cereals	.020	-.032	-.084	**.291****	.123	.127	**.272****	**.273****	**.368****	-.089	1	.097	**-.219***
	.825	.725	.366	.001	.171	.155	.002	.002	.000	.323		.278	.014
Non water drinks	.062	.045	-.151	**.472****	**.276****	-.103	**.191***	.060	**.249****	-.122	.097	1	**-.273****
	.488	.614	.103	.000	.002	.253	.032	.505	.005	.173	.278		.002
Total DVS	**.293****	**.255****	**.280****	**-.445****	**-.496****	.085	**-.423****	**-.216***	**-.387****	**.231****	**-.219***	**-.273****	1
	.001	.004	.002	.000	.000	.343	.000	.015	.000	.009	.014	.002	

4.5.3 Principal component analysis

Principal component analysis (PCA) was undertaken to determine if there was a clustering of dietary characteristics that defined the participants into groups, irrespective of dietary exclusion status. Prior to performing PCA, the suitability of data for factor analysis was assessed. Inspection of the correlation matrix revealed presence of many coefficients of 0.3 and above. The Kaiser Meyer-Olkin value was 0.78 with a Bartlett Test's of Sphericity of $p = 0.00$ indicating PCA was appropriate.

A number of continuous variables were used in the PCA analysis. Variables were chosen to be inputted into the analysis based on the existing literature on infant feeding. PCA revealed the presence of four components with eigenvalues exceeding 1, with these four components explaining 26.8%, 14.1%, 13.7% and 8.1% of the variance respectively and 62.9% of the variance cumulatively. Examination of the scree plot indicated a break after the fourth component. Using the scree test, these four components were retained for further investigation.

To aid in the interpretation of these components, varimax rotation was performed. The rotated solution showed all components had a number of strong loadings and most variables loaded substantially on only one component. Details are shown in Table 4.14. The first cluster was labeled as the "milk-free' and includes a long duration of substitute formula use, exclusion of more foods, lower consumption of dairy products, higher consumption of soya products and readymade baby food. Cluster two, labeled as "problem eaters" is characterised by higher levels of fussy eating, food neophobia and feeding difficulties. Cluster three, labeled "mixed diet" is characterised by more frequent consumption of cereals, meat/fish, sweet foods/miscellaneous foods, fruits and vegetables. Cluster four, labeled as "healthy" is characterised by a longer duration of breastfeeding, a higher awareness of healthy eating and also scored relatively highly on fruit and vegetable consumption.

Following the PCA analysis, each participant's score for each cluster was saved as a variable. A Mann Whitney test was undertaken to determine if there was a difference in cluster scores between participants in the CME and control groups. Significant differences were found for the first cluster "milk-free", ($p < 0.01$) but not for any of the other three clusters; "problem eaters", "mixed diet" or "healthy" ($p = 0.199$, $p = 0.068$ and $p = 0.303$ respectively). Correlation analysis was undertaken to determine if the child's age or maternal age was associated with any of the clusters. Age of child was found to be strongly positively correlated with the "mixed diet" group ($rho = 0.578$, $p < 0.01$), meaning older children scored more highly with this pattern than younger children. A higher maternal age was found to be moderately positively correlated

to the “healthy” cluster (rho = 0.296, p = 0.02), meaning older mothers scored higher on this pattern.

Table 4.14 Principal component analysis variables

Variables	Component			
	Milk-free	Problem eaters	Mixed Diet	Healthy
Duration of substitute formula	**.858**	.218	.079	-.037
Dairy product intake	**-.834**	-.118	.359	-.002
Number foods excluded	**.807**	.137	-.117	.123
Soya product intake	**.682**	.137	.171	.055
Readymade baby food intake	**.483**	-.064	-.268	-.143
Fussy eating score	.106	**.940**	-.029	-.059
Food neophobia score	-.134	**.874**	.024	.050
Feeding difficulty score	.163	**.874**	-.067	-.023
Meat and fish consumption	-.084	-.017	**.710**	-.055
Starchy/cereal food consumption	.130	.070	**.673**	.061
Diet Variety Score all foods	.307	.268	**-.663**	.001
Sweet /miscellaneous food consumption	-.316	.106	**.636**	-.333
Fruit consumption	-.065	-.155	**.556**	.513
Vegetable consumption	.121	-.227	**.500**	.486
Breastfeeding duration	-.300	-.024	-.016	**.700**
Attention to healthy eating	.304	.032	-.146	**.616**

Extraction Method: Principal Component Analysis. Rotation Method: Varimax with Kaiser Normalization. Rotation converged in 5 iterations.

4.6 Discussion

This study set out to compare the eating habits of two groups of young children; one group consuming a CME diet for diagnosed or presumed CMA and one group consuming an unrestricted diet. There were two primary objectives set for this study, for which it was sufficiently powered:

1. To determine the degree of fussy eating, feeding difficulties and food neophobia in infants and toddlers consuming a CME diet, compared to a control group.
2. To determine dietary variety in infants and toddlers consuming a CME diet compared to a control group.

Overall the CME group scored significantly higher on all three measures of fussy eating, feeding difficulties and food neophobia, although all of these variables were within normal ranges. All

three outcomes were significantly positively correlated with a higher incidence of allergic symptoms. Participants in the CME group overall had lower dietary variety, which was mainly attributed to differing intakes of dairy/egg products, readymade baby foods and sweet/miscellaneous foods. A secondary objective of the study was to determine the growth status of young children consuming a CME diet, compared to a control group. There was no difference found between the two groups for any anthropometric measurement, however the study was not sufficiently powered to detect a difference.

4.6.1 Infant feeding characteristics compared to national infant feeding practices

Overall this group of participants' infant feeding habits are broadly reflective of infant feeding habits observed nationally and of previous studies in this geographical area (Venter et al., 2009). The current breastfeeding initiation rate in the UK is 81% (Mc Andrew et al., 2012), however the prevalence of breastfeeding declines rapidly with increasing infant age, with only 34% of infants still being breastfed at six months old. Nationally introduction of formula occurs in 67% of infants between the age of 4-10 weeks and by 7-10 months old, 89% of infants have been fed some formula milk. A previous food allergy study conducted on the Isle of Wight in 2001-2002 indicated that 66% of infants were breastfed at one week old, falling to 12% at one year old, with formula being introduced at a median age of 14 days (Venter et al., 2009). In this study 91.3% of participants had been given formula milk, with greater use of formula observed in the CME group than the control group (97.0% and 82.3% respectively). This finding may be influenced by the difficulty mothers experience adhering to a CME diet themselves whilst breastfeeding. A recent Cochrane review on maternal dietary exclusion during pregnancy and lactation concluded that adherence to an antigen avoidance diet during lactation requires considerable effort and more information is needed about women's experience and compliance with such diets (Kramer & Kakuma, 2014).

In terms of introduction of solid food, nationally 30% had introduced solids by four months old and 75% by five months old (Mc Andrew et al., 2012). This study found a median age of introduction of solids of 20 weeks, with those in the CME group introducing solid, lumpy and finger foods significantly later than those in the control group. This may be due to anxiety of introducing solid food to a child who has already had an adverse reaction to formula or breast milk, a phenomenon known as "reverse causality" (Grimshaw et al., 2013) or perhaps weaning is delayed slightly whilst waiting for an appointment with a health professional before commencing the process.

A large food allergy birth cohort study in Germany (Schoetzau et al., 2002) found similar findings in relation to eczema, reporting that mothers of infants with eczema had delayed solid food introduction beyond six months old. In contrast, in a large birth cohort study from a similar geographical location as this PhD study, solids were also introduced at 20 weeks, but they were introduced significantly *earlier* in food-allergic infants compared with control infants (Grimshaw et al., 2013). This was attributed to the fact that parents of allergic children were happier to introduce these foods due to having had a SPT, which the control group of children did not have. However, data collection for Grimshaw et al's study took place 10 years ago and during this time lapse, allergy weaning guidelines have been debated considerably (Fleischer et al., 2015; Muraro et al., 2014). In this PhD study data on introduction of solids was collected retrospectively, so may be subject to recall bias. Nevertheless, UK Department of Health weaning guidelines for the general population suggest introduction of solids to occur "around the age of 6 months", which both groups did not fully adhere to. Choice of first food was similar to that seen nationally with 51% of all participants choosing baby rice, compared to 57% in national surveys (Mc Andrew et al., 2012). Specific information on the age of introduction of allergenic foods was not collected, as this was not a focus of the study.

Infants in the CME group excluded cows' milk from their diet relatively early (median age 9.5 weeks). This could be due to the relatively short waiting list at the clinic where the study took place, however this is only speculation. In support of this opinion, previous research has indicated that confirmation of a diagnosis of CMA and treatment with substitute formula can take several months of repeated visits to the GP. A UK study (Taylor, Sladkevicius, Panca, Lack, & Guest, 2012) reported that the mean time to commence a substitute formula for CMA was 2.2 months after the initial visit to the GP, with the diagnosis of CMA not documented for a further few weeks. The waiting time to see a paediatrician was a further 2.5 months.

The proportion of participants in the CME group consuming an AAF (Neocate LCP) (45.5%) was considerably higher than would be expected in a secondary care allergy clinic. By definition, an EHF should be tolerated by 90% of infants with CMA, therefore an AAF should only be required by 10% of infants with CMA (Høst et al., 1999; Venter, Brown, Shah, Walsh, & Fox, 2013). As described in chapter two, several national and international guidelines advise that an EHF should be used as first line treatment in the infants with CMA, except for those infants presenting with severe symptoms (Fiocchi et al., 2010;Venter et al., 2013). In this group of infants presenting with symptoms suggestive of CMA, 27% of the sample were initiated on an AAF, without first using an EHF. However, this should not necessarily be interpreted that there was a higher than expected proportion of infants with severe CMA symptoms requiring

an AAF. Indeed, it is most likely a reflection of the fact that prescribing practices differ depending on which health professional first assessed the infant and their knowledge of CMA. Knowledge of guidelines of food allergy by primary care staff in the UK is thought to be suboptimal (Walsh, 2014).

4.6.2 Symptoms reported by respondents

The symptoms reported in this sample of infants with presumed CMA are typical and characteristic of CMA (NICE, 2011). A previous study reported that 44% of infants who went on to receive a diagnosis of CMA originally presented with eczema and gastrointestinal symptoms and < 5% presented with urticaria, which is similar to this study's findings (Taylor et al., 2012). The fact that those with a history of maternal food allergy reported more symptoms may be due to (a) heredity of atopic conditions or (b) increased maternal anxiety/awareness of potential symptoms. However, the fact that there was no link with paternal food allergy or sibling food allergy, discounts the heredity element to a certain extent. Previous research conducted in this geographical area indicated that mothers with atopic history were more likely to report a food-related problem in their child at six months and two years old than mothers without an atopic history (Venter et al., 2009).

Although some of the allergy history questions were taken from an established questionnaire (Asher et al., 1995), reporting of symptoms may be highly influenced by the interpretation of the question (e.g. the understanding of the definition "wheeze" may vary between individuals). Additional questions regarding gastrointestinal symptoms were also included in the questionnaire (vomiting, diarrhoea, constipation, colic and abdominal distension). Again, it is possible that interpretation of these symptoms will vary between individuals.

4.6.3 Differences according to SPT status

All participants from the CME group had a SPT as part of routine clinical care. Although clinical history, SPT and exclusion/reintroduction of cows' milk were used to diagnose CMA and to distinguish between IgE and non IgE CMA, it is acknowledged that this method has limitations. It is possible that infants with a positive SPT may have been sensitised, rather than clinically allergic and therefore misclassified as IgE CMA rather than non IgE CMA. Additionally some participants may have had mixed IgE and non IgE CMA. Therefore although the terms "IgE" and "non IgE" may be used in this thesis when referring to the positive and negative SPT groups respectively, they are used with this caveat.

The differences in symptoms according to SPT status was characteristic of the

differences typically seen between non-IgE and IgE mediated CMA (Høst & Halken, 2014; NICE, 2011). However, the finding that the infants with a positive SPT had significantly lower weight/age than those with a negative SPT is interesting, especially as it was not influenced by the number of foods avoided. BMI was not affected; however complete length/height data was not available for all participants, as it can be difficult and inaccurate to measure in this age group. Some previous studies investigating differences in growth status between food avoidance and control groups, have not reported on SPT status (Christie et al., 2002; Flammarion et al., 2011) or else only recruited participants with non-IgE CMA (Vieira et al., 2010). A multicentre UK study of food allergic children did stratify results by IgE status, but did not report a statistical difference between growth parameters between the IgE-mediated, non-IgE-mediated and mixed IgE groups (Meyer, De Koker, et al., 2014). However, that study included children with a range of food allergen avoidance diets, and the participants were slightly older (mean age 27 months), therefore the results are not directly comparable. A systematic review (Sova et al., 2013) concluded that the heterogeneity of paediatric food allergy population makes research difficult and that categorising children by type of allergy may be superior to dividing by number of food allergies.

It is possible that the difference seen in weight centile between SPT groups may be due to the more strict exclusion of high calorie snack products containing traces of cows' milk in those with IgE mediated CMA (Mehta, Groetch, & Wang, 2013). Looking at the actual weight centile figures, both median values are within the normal range (45.3% and 70.4%); therefore although the difference is statistically significant, the clinical relevance may not be as important. In addition, both SPT positive and SPT negative groups had infants in the extreme ranges of weight/age centile (5.6-137%), indicating that a wide spectrum of weight/age centiles is present in this population. Growth status will be discussed further later in this discussion section.

A further difference seen between the two groups per SPT status was the later introduction of substitute infant formula in those with a positive SPT (20 weeks and 8 weeks respectively). This could be because typical non-IgE symptoms such as colic and reflux tend to present very early in infancy (Lucassen et al., 2001), therefore parents may seek advice and be referred for CMA investigations earlier. In support of this hypothesis, it has been reported that adverse reactions to food presenting with gastrointestinal symptoms (characteristic of non-IgE mediated allergy) tend to cause parents more emotional worry and concern than skin and respiratory symptoms (Merras-Salmio et al., 2013).

4.6.4 Feeding difficulties

The higher feeding difficulty scores observed in the CME group compared to the control group was statistically significant. This is the first time this has been reported in a study of infants with suspected food allergy using a control group and a validated questionnaire (Ramsay et al., 2011). However it should not be overlooked that both groups had median scores well within normal levels (< 45). Indeed the number of children in the control group with feeding difficulties (1.6%) is considerably lower than that reported in previous studies of normal healthy developing children (Crist & Napier-Phillips, 2001; Wright et al., 2007), however the methodology for those studies was different and perhaps the questionnaire used in this study is more sensitive. A significant difference was also found in feeding difficulty score between participants with and without a maternal history of food allergy, which may be due to a heightened awareness of problems with food in their offspring.

Previous research has established links between the presence of gastro-oesophageal reflux disease and feeding difficulties (Mathisen, Worrall, Masel, Wall, & Shepherd, 1999) although that study did not mention food allergy. Similarly, a link has been established between colic and feeding difficulties, however the authors were unable to conclude whether colic and feeding difficulties were co-existing problems with a similar aetiology, or whether one caused the other (Miller-Loncar, Bigsby, High, Wallach, & Lester, 2004). As already explained, studies of feeding difficulties and food allergy have typically been conducted on children with complex gastrointestinal (non-IgE mediated) allergies (Meyer et al., 2014; Mukkada et al., 2010; Wu et al., 2012) or in children who also have an underlying comorbidity (Pentiuk et al., 2007), therefore the participants are not necessarily reflective of the “average” infant with suspected CMA.

Meyer’s study (n = 437) (2014) found that 30-40% of children with Food Protein-Induced Gastrointestinal Allergies (FPIGA) had feeding difficulties reported in their medical notes, with a higher rate in those with symptoms of abdominal pain, vomiting, bloating and constipation. However as previously explained, Meyer’s study recruited a different population to this study group: the disease process is more complex and the participants were older, meaning there is more time for dysfunctional eating habits to become ingrained. The study took place at a regional specialist children’s hospital, therefore it is not surprising the rates of feeding difficulties were so high. Although there are many differences between Meyer’s study and the present study, there are some common findings. i.e. the present study also identified significant correlations between colic, vomiting and constipation and feeding difficulty score. Interestingly, Meyer et al. (2014) identified a significant correlation between feeding difficulties and extra-

intestinal manifestations (joint pain, lethargy, night sweating and headaches). Although the present study did not specifically collect data about these symptoms, a significant correlation was found between other non-gastrointestinal symptoms (wheeze and cough) and feeding difficulty score. This serves to illustrate that eating and feeding habits in childhood are influenced by a wide range of health-related symptoms that are not necessarily gastro intestinal.

In the present study, feeding difficulty score was not found to be related to socioeconomic status (parental education/occupation) or birth order/number of siblings, which is in agreement with Crist & Napier Phillips findings (2001). However contrary to previous research (Northstone et al., 2001), a link between the age of introduction of any type of solid foods and feeding difficulty score was not identified. Introduction of lumpy foods did contribute to the multiple regression model predicting higher feeding difficulty score, but only in combination with other variables. However it must be highlighted that the reporting of age of introduction of solid food was based on parental recall, which may affect the accuracy of this data. Also as only 10 participants (7.9%) from the whole sample had scores diagnostic of clinical feeding difficulties, perhaps the relatively small sample size may have been unable to detect a real correlation. Again, differences in methodology cannot be ignored as the measurement of feeding difficulties in Northstone's study was assessed using only a few questions with a yes/no answer.

A higher number of dietetic contacts was correlated with increasing feeding difficulty score, as was being concerned with healthy eating. This could be explained by the fact that infants with feeding difficulties may cause parental anxiety, leading parents to seek more support and help from health professionals. Parents who are more concerned with healthy eating may perceive more feeding difficulties in their child if the child refuses to consume the specific foods the parent offers. It must be made clear however, that all of these are simply correlations, rather than "cause and effect" and none of the correlations could be considered strong (rho > 0.5). In addition, the direction of the regression or correlational relationship is not known. It may be that those who present with feeding difficulties are not given lumpy foods due to fear they will choke, rather than delaying the introduction of lumpy foods causes/worsens the feeding difficulties.

4.6.5 Fussy eating

Overall infants in the CME group scored significantly higher on the fussy eating questionnaire than the control group (22.5 and 18 respectively). However the median score of 22.5 is still very far below the maximum questionnaire score of 70, indicating that as a whole the group were

not particularly fussy eaters. Due to differences in scoring subscales of the questionnaire it is not possible to directly the compare the scores with other studies. It is also not possible to classify the proportion of children who were “fussy eaters” or “non-fussy” eaters as the scale used measures a continuum of fussy eating behaviour, rather than a binary outcome.

The most similar study to have used this questionnaire involved 12 month old infants in Ireland, which examined fussiness in relation to texture of foods (Blossfeld et al., 2007). The authors found a mean score of 25 on a subscale of the questionnaire, which is not too dissimilar to the present findings. They had a similar proportion of infants who were currently being breastfed (15.7%), reporting that those infants who were breastfed were less fussy with regard to textured carrots. Other studies have also found that a longer duration of breastfeeding is linked to less food fussiness (Galloway et al., 2005; Shim, Kim, & Mathai, 2011), the hypothesis being that breastfeeding provides an opportunity for flavours to be transmitted via the mothers’ milk compared to the uniform flavour of infant formula (Beauchamp & Mennella, 2009). This study did not find a link between breastfeeding duration and fussiness in either group. However, the fundamental difference is that the previous studies did not include infants fed substitute formula for CMA, which have an altered taste. As discussed in previous chapters, it may be that the bitter taste of substitute formula leads infants to have altered taste preferences for a broader range of foods. However, within the CME group, we did not find any correlation between scores for fussy eating and duration or type of substitute formula use.

Weak, but statistically significant correlations between colic, wheeze and cough and fussy eating scores were identified, and a moderate correlation between number of allergic symptoms and fussy eating score, but no correlation for constipation, vomiting, eczema or abdominal distension. As is the case with feeding difficulties, the direction of the correlation is not known. A recent large scale study of four year old children in Holland identified a bidirectional correlation between constipation and fussy eating (Tharner et al., 2015). They identified a subset of the cohort had history of CMA, but there was no difference in fussy eating levels between those with and without CMA history (personal communication Tharner, January 2015).

Previous research has reported that fussy eating is pervasive across different countries, socioeconomic statuses, genders, ethnic groups and ages (Carruth et al., 1998; Dubois et al., 2007; Goh & Jacob, 2012; Taylor et al., 2015; Tharner et al., 2014; Xue et al., 2015). Across all participants, we did not detect a difference in fussy eating score in relation to parental education or occupation status. This is in contrast to some studies which have reported higher levels of fussy eating in higher socioeconomic groups (Goh & Jacob, 2012; Tharner et al.,

2015). We also did not find any association between fussy eating and maternal age. A caveat to this statement is that *maternal* levels of fussy eating or parental strategies used to deal with fussy eating were not measured. As previously discussion in the literature review chapter, it is known that parents of picky eaters use different strategies than those who are not picky, which may compound the problem (e.g. the child may be offered a less varied of diet as they have already been labelled "fussy"). It also is known that parents only offer their child a given food a finite (and often insufficient) number of times if it has been rejected previously (Carruth et al., 2004).

Unfortunately the regression model to predict fussy eating score was relatively weak, only accounting for 21.4% of the variance. However this simply highlights the fact that infant feeding behaviour is multifactorial in origin and difficult to predict. It is notable that the total volume of milk/milk substitute consumed/day made the most contribution to this model and was the only variable to make a unique statistically significant contribution ($B = 0.258$, $p = 0.039$). In Wright et al.'s (2007) study of healthy children, "preferring drinks to food" was one of the most common problems perceived by parents and the number of milk drinks per day was inversely correlated with appetite. Using the strategy of 'giving the child milk from a bottle' was associated with persistence of fussy eating in a study of children aged 1-10 years in Singapore (Goh & Jacob, 2012). Furthermore a recent study of 16 month old twins in the UK reported that a higher intake of formula is associated with picky eating behaviour, as the formula acts as a substitute, rather than a supplement to food (Syrad, van Jaarsveld, Wardle, & Llewellyn, 2015). This supports the simple anecdotal advice that is frequently given to parents of fussy eaters by dietitians to reduce excessive consumption of formula in order to encourage a better appetite and mealtime behaviour.

As previously discussed in an earlier chapter, fussy eating is thought to affect nutritional intake to some extent (Galloway et al., 2005) and it has been suggested that fussy eaters tend to consume more sweetened foods (Carruth et al., 2004), less vegetables and less fish (Cardona Cano et al., 2015; de Moor et al., 2007; Jacobi et al., 2003; Tharner et al., 2015). In this study, significant correlations were reported between increased fussy eating and soya/substitute products ($rho = 0.204$) and an inverse correlation with dairy/egg products ($rho = -0.203$); however both of these are confounded by the CME group having a greater number of allergy symptoms, which is directly correlated with fussy eating. In addition, a significant inverse correlation with fussy eating and vegetable consumption ($rho = -0.196$) and overall dietary variety was found ($rho = 0.293$), but no correlation with fruit, meat, cereals or sweet/miscellaneous foods. The correlations were weak-moderate, which can probably be

attributed to the fact we did not observe very high questionnaire scores for fussy eating. Likewise a correlation was not observed between fussy eating score and any growth measurement, contrary to some previous research (Dubois et al., 2007; Ekstein et al., 2010; Tharner et al., 2015; Wright et al., 2007), but in agreement with one study (Jacobi, Schmitz, & Stewart Agras, 2008). As nutritional intake was not measured, it is impossible to say whether the reduced dietary variety and vegetable intake would have a noticeable nutritional or clinical effect.

4.6.6 Food neophobia

Although only 92.9% of participants completed the food neophobia questionnaire fully, this still remains an excellent response rate. The discrepancy compared to the 100% completion of the other questionnaires can be explained by the younger age of participants and perceived irrelevance of some of the questions. Food neophobia scores were strongly correlated with both feeding difficulty and fussy eating scores (rho = 0.830 and 0.795, $p < 0.01$) therefore it is not surprising that similar differences between control and CME groups were seen, with similar factors correlated. Very few infant feeding, socioeconomic or allergic factors were correlated with food neophobia score in this group of participants. This may be because food neophobia typically peaks between the ages of 2-6 years old, whereas the median age of participants in this study was 13 months. However, as previously mentioned, most CMA resolves by the age of two years old (Schoemaker et al., 2015), therefore it would be more difficult to perform this study in older children. Because so few studies have measured food neophobia in children under two years old, it is difficult to compare the results. For example, the original validation study of the questionnaire had 5-6 year old participants recording mean scores of 38-42 (depending on gender) (Pliner & Loewen, 1997), compared to the median score of 21 reported here. A more recent study of 2-5 year old children reported mean scores of 36 (Russell & Worsley, 2008). Overall the lower scores measured in this study are probably due to the fact that food neophobic behaviours have not yet manifested in this young group of participants. Therefore the statistically significant difference we reported between the CME and control group, should not be over interpreted and ideally needs to be re assessed at an older age.

No difference in food neophobia score according to breastfeeding status or duration or age of introduction of solid foods was seen. Other studies have not reached a consensus on whether early food exposure has a positive or negative affect on childhood food neophobia (Howard et al., 2012; Russell & Worsley, 2008). Similarly, previous research has disagreed the extent to which maternal/sibling neophobia contributes to child food neophobia (Cooke et al.,

2007; Pliner & Loewen, 1997). As maternal food neophobia was not measured, it is not possible to speculate. However, unlike the correlation seen with feeding difficulty score, a correlation between maternal food allergy history and child food neophobia was not found. This implies that a mothers' history of an adverse reaction to a food does not influence her infant's willingness to try new foods. In terms of effect on food consumption, as was the case with fussy eating score, a weak, but statistically significant inverse correlation with vegetable consumption (rho = 0.210) and overall dietary variety (rho = 0.280) was identified, in addition to correlations with dairy/egg and soya/substitute foods, but no correlation with fruit, meat/fish, cereal or sweet/miscellaneous foods. Although the correlation scores reported are weak, they are similar in magnitude to previous studies of food neophobia (Cooke et al., 2003).

4.6.7 Growth

Although a significant difference was seen in weight status between SPT negative and SPT positive participants within the CME group, no difference in any growth measurement was seen between the CME and the control group. It has been reported elsewhere that growth of allergic children with CMA is impaired compared to that of non-allergic children up to the age of two years (Agostini et al, 2007; Isolauri et al, 1998), which is thought to be related to dietary restrictions and/or the underlying pathophysiology of the allergic disorder (Vieira et al, 2010).

A recent study of patients with suspected food allergies from general paediatric practice in the US reported that children under two years old consuming CME diets did not experience weight impairment. The authors attributed this to the fact that prescribed formula consumed by children under two years of age provides adequate nutrition to compensate for the exclusion of cows' milk products. However in older children, a substitute formula is not prescribed and the alternative milks used are typically lower in calories and protein. The authors also identified that many typical toddler snack foods contain milk and may be avoided. A difference in growth status between single and multiple exclusion diets was not found (Mehta et al., 2014), which concurs with the findings of Berry et al (2014). Although Mehta et al.'s study is in a different continent with differing food patterns, culture and medical services, it is one of the few studies to also have been undertaken in a primary care population. Similar to the present study, the children were following the exclusion diets for presumed or physician-diagnosed food allergies, rather than challenge-proven food allergies, meaning the population is similar and typical in many ways.

It is also worth highlighting that participants in the CME group all had dietetic consultations (median three contacts), meaning they would have received individualised

nutritional advice and growth monitoring at timely intervals. Referral to a trained dietitian is recommended in UK national guidelines (Luyt et al., 2014; NICE, 2011) and it has been shown to improve nutritional outcomes (Berni Canani et al., 2014; Christie et al., 2002), but not specifically feeding difficulties. As already previously discussed, the participants in this study commenced a substitute formula at a young age of 11 weeks and did not have a long waiting time to be seen by a paediatrician and dietitian, meaning any growth deficits could be addressed promptly. The growth results of this study cannot therefore be compared to studies of unsupervised CME diets.

4.6.8 Interpretation of food frequency questionnaire results

The FFQ analysis identified differences in both frequency of consumption and variety of consumption between CME and control groups. Differences in frequency and variety of the diet will now both be discussed. Whilst some of the differences between groups are logical and expected, other differences are more revelatory and will be discussed in more detail, particularly the consumption of readymade baby food.

4.6.8.1 Frequency of consumption of different food and food groups

Differences observed between the two groups in consumption of dairy/egg and soya/substitute food categories are to be expected in a study of this nature and are self -explanatory. Likewise, lower consumption of breakfast cereal in the CME group is probably because it is a food that is typically served with milk. Lower consumption of orange and banana in the CME group is likely because certain fruits are potentially or perceived to be allergenic (Zuidmeer et al., 2008). Lower consumption of the sweet/miscellaneous foods category could be attributed to the fact a lot of these foods contain milk (e.g. biscuit and ice-cream), or possibly due to the higher concern with healthy eating and more dietetic contact the CME group have had.

Looking at consumption of beverages, the “healthy eating” aspect may also explain the less frequent consumption of non-water drinks (e.g. tea, baby juice) in the CME group. Research in children aged 1-5 years suggests that milk intake is inversely related to consumption of juice drinks and added sugar beverages and that consumption of high sugar beverages is inversely related to dietary quality. (Marshall, Eichenberger Gilmore, Broffitt, Stumbo, & Levy, 2005). Although this study did not find a statistically significant difference in the volume of consumption of cows’ milk formula/substitute formula between groups, this may be because the majority of the participants (115/126) were under two years old and most participants in the CME group were still being prescribed a substitute formula. Once children with CMA are over two years and substitute formula is no longer prescribable, it may be that

intake of sugary drinks increases. This would concur with the theory that children with a restricted diet develop a strong preference to calorie-dense “safe” foods resulting in increased juice consumption (Somers, 2008).

4.6.8.2 Dietary variety differences between groups

Overall, the CME group was found to have a significantly less varied diet than the control group. This was the case whether dairy/egg/soya substitutes were included or excluded from the calculation. Amongst food subcategories the CME group had a less varied intake of dairy/egg/soya substitutes, meat and sweet/miscellaneous foods and a greater variety in the readymade baby food category. Whilst it may be expected that the CME group have a less varied diet overall and a less varied intake of the dairy/egg foods, the lower variety in the meat and sweet/miscellaneous categories are of more interest. It is perhaps an indication that parents are over-restricting the diets of children with CMA, or it may be a reflection of the ubiquity of milk in processed foods. For example, within the meat category there are items such as ham and fish fingers, which may contain small quantities of milk, and within the sweet/miscellaneous category there are items such as biscuits and cakes, which are highly likely to contain milk. It does not appear that children consuming CME diets are fed a greater variety of other food categories (e.g. fruit, vegetables, or starchy carbohydrates) to compensate for the restriction of dairy products.

Dietary variety in food allergic children has not been specifically investigated to date. One study was identified that measured “dietary monotony” by means of a parent-report questionnaire in an Italian study of mothers of food allergic children age 0-16 years (n = 124) (Polloni et al., 2013). The majority of the children in the study were under five years old and consuming a diet excluding more than one food item. Most of the participants claimed to have a “monotonous diet”. When asked about causes of the repetitive diet, the responses were: strict avoidance, low curiosity about food, a limited choice of food industry safe products and difficulties in making traditional recipes. Similar to this study’s findings, Polloni et al. (2013) also found an inverse association between child age and the repetitiveness of the diet. They hypothesised this was due to children outgrowing some food allergies, or that the diet becomes more varied as families become more accustomed to available food products and dietary restrictions. However Polloni’s study had several limitations in that the questionnaire was not validated, there was no control group, no dietary data was reported and only children with IgE food allergies were included.

4.6.8.3 Differences in consumption of readymade baby foods between groups

There were significant differences in both the frequency and variety of consumption of readymade baby foods between groups. The CME group ate readymade baby food significantly more often than the control group and ate a greater variety of readymade baby food than the control group. The FFQ listed nine different types of readymade baby foods, including cereals, fruit puree, desserts, meat/fish based meals and vegetable/pasta/rice savoury meals. In total, these foods were eaten 15 times more frequently by the CME than the control group (thirty times per month and twice per month respectively) ($p < 0.01$). This is a novel finding and it is particularly interesting that the difference was most pronounced in children greater than one year old, as typically readymade baby food products are intended for the initial stages of weaning. Indeed a national infant feeding survey in the UK indicated that the use of readymade baby foods in the general population was most common between the ages of five and ten months (Mc Andrew et al., 2012).

Consumption of readymade baby food is increasing and qualitative research has indicated that it is perceived as potentially "safer" and composed of superior ingredients by some mothers (Maslin et al., 2015 in draft). This may explain the higher consumption of these types of foods seen in the CME group. It is known that consumption of food outside the home is increasing (Jabs & Devine, 2006). Readymade baby food is perceived as more convenient and portable (Caton, Ahern, & Hetherington, 2011), therefore it may be that infants with CME are fed these products as it is difficult to source guaranteed cows' milk free meals and snacks when eating away from the home.

Previous research using a case control study design, has reported that reported that a diet higher in fruit, vegetables and homemade foods, and lower in commercial baby foods was associated with a reduced prevalence of food allergy (Grimshaw et al., 2014). The authors hypothesised that this pattern may be a contributing factor to the development of food allergy, rather than be a result of having a food allergy, stating that children with food allergy are more likely to consume home prepared foods because they are safer. The present study clearly shows that those in the CME group consume readymade baby food *more* frequently than the control group. Although the data generated from the present study is cross sectional and causation cannot be inferred in either direction, it is likely that increased consumption is a result of the CME diet. Given that the two studies took place approximately seven years apart, it is possible that there is currently a greater availability of milk free baby foods on sale.

Irrespective of whether the consumption of readymade baby food contributes to the development of food allergy, or is a result of it, there may be long-term nutritional implications.

Commercially produced infant food has a different taste profile and nutritional content to homemade food. Garcia et al. (García et al., 2013) compared homemade baby food to four hundred commercial baby food products available in the UK. The study confirmed that the nutritional quality of homemade baby food is generally superior; with the exception of rusks and biscuits, which were higher in iron and calcium than homemade versions, although also higher in sugar. The majority of products were found to be sweet, with a distinct lack of bitter vegetables used (Garcia, McLean, & Wright, 2015). Additionally, a study from the USA reported that many types of commercial infant and toddler foods had equivalent levels of sodium and sugars to products aimed at older children or adults (Cogswell, Gunn, Yuan, Park, & Merritt, 2015)

A further disadvantage of commercial baby food is that the microbial load is negligible due to food safety requirements, meaning children who consume a diet high in commercial baby food may be exposed to a lower microbial load. The role of the microbiota in the development or allergic disease has been researched for some time, with data indicating differences between the gut bacteria of allergic and non-allergic infants (Björkstén et al., 2001).The present study did not investigate the role of microbiota in CME diets, but it is worth considering. To summarise, this study has identified a novel trend in the consumption of readymade baby food in older infants consuming a CME diet, which is a growing area of research that requires further investigation.

4.6.9 Characterisation of participants using principal component analysis

The four distinct groups identified by PCA explained a large proportion of the variance (62.9%). These four groups provide an interesting perspective on the entire study, by identifying key differences and similarities amongst the participants. The “milk-free” cluster is the most defining of the four groups, explaining 26.8% of the variance and it is very interesting that a higher consumption of readymade baby food scores most highly with the cluster. The second cluster, labeled as “problem eaters”, explained 14.1% of the variance, was notable as none of the other food related factors score heavily on this cluster. It was not found to be related to either age of the child or maternal age, supported by research indicating that feeding problems are widespread across all demographics (Crist & Napier-Phillips, 2001; Wright et al., 2007). The third cluster, strongly correlated with increasing age of the child, was characterised by a mixed diet, with more fruit, vegetables, meat, cereals and sweet foods. This reflects the reality that infants and toddlers start to have more diversified and “adult” like food intakes as they grow older. Finally the fourth cluster, which explained 8.1% of the variance, was characterised by

breastfeeding duration and awareness of healthy eating. This cluster was correlated with a higher maternal age. Previous research has reported that infant feeding guidelines are more likely to be followed by older mothers (Moore et al., 2014; Robinson et al., 2007).

PCA has been used in a number of infant dietary studies, with the advantage being that it can identify underlying food *patterns* (Golley et al., 2012; Grimshaw et al., 2014). Foods are not consumed independently from each other, therefore should not be analysed separately. Consumption of food groups are often interrelated, with a greater consumption of one food group leading to a greater/lesser consumption of another or vice versa. This was illustrated in this study by the positive correlation between sweetened foods and non-water drinks ($r = 0.472$). However, a disadvantage of PCA is that it is reliant on the subjective assessment of input variables, which may be influenced by the researchers' own preconceived opinions on what patterns will or should cluster together. In addition, it is possible that PCA may not uncover all patterns in the dataset, as only those selected by eigenvalue are chosen. Other studies using PCA with FFQ data have entered individual food items into the matrix, rather than food groups, as used in this study, which perhaps would have revealed slightly different clusters. However, due to the number of participants (126) and the large number of individual foods in the FFQ used (89), the PCA calculation using individual foods would not have been robust.

4.6.10 Limitations

There are a number of limitations to this study. Firstly there may be a recruitment bias whereby those more interested in diet and nutrition or are more likely to take part, although all eligible participants in both groups were approached to help minimise this bias. This is a largely unavoidable problem of many nutrition research studies. However the fact that participants had a broad range of demographic characteristics suggests that the sample is representative of the population from which it was recruited. The questionnaire method used is reliant on parental report, which is subjective, rather than observation of infant feeding, which would have been more objective. However, the environment used in laboratory studies of mealtimes is artificial and the video analysis methodology is labour intensive and impractical. The control group was slightly older than CME group. As food consumption patterns change with age, it is possible that this may have skewed the results slightly. Attempts were made to overcome this by stratifying some statistical analyses by age. The CME group was heterogeneous, in the sense that it included participants consuming both single and multiple exclusion diets.

Most crucially, as this was a typical caseload of patients from a secondary care allergy clinic, rather than a randomised control study, we did not have diagnostic confirmation of CMA

in the CME group, or the classification of IgE or non-IgE CMA. None of the participants had undergone a food challenge; instead they had physician-diagnosed CMA using clinical history, SPT and dietary exclusion/reintroduction. As previously mentioned, participants were recruited using consecutive sampling from clinic. They were not randomised to consume a particular formula and were not necessarily prescribed the most appropriate EHF/AAF based on guidelines of severity of symptoms. It is therefore is not possible to conclusively separate the effect of the disease from the treatment (CME diet). However the reality is that there are not sufficient resources to undertaken food challenges in all infants with suspected CMA and some infants may be consuming a CME exclusion diet unnecessarily. In awareness of this, caution has been exerted throughout the study to refer to the CME group as a diet exclusion group, rather than a food allergic group.

4.6.11 Strengths

The strengths of this study are the use of a control group, which was recruited from the same geographical locality as the CME group, allowing direct comparison. The groups were closely matched for all demographic variables, except participant age, which differed by only three months. As the research took place in a secondary care allergy clinic, the results are broadly generalisable to the majority of other clinics around the UK. The fact that the infant feeding data of the group as a whole is so similar to national feeding trends demonstrates that the control group is also reflective of the general population.

The recruitment target of the study was met, meaning the study was sufficiently powered to investigate all the primary outcomes and was of similar size to several published studies. All the questionnaires that were used were specific to the age group and validated. All data collection, coding, analysis and interpretation took place by the same researcher to minimise the effect of researcher bias.

4.7 Conclusion

In summary, it has been demonstrated that infants consuming a CME diet have significantly higher scores of feeding difficulties, fussy eating and food neophobia than a control group consuming an unrestricted diet. This may be due to the underlying disease process resulting in allergic symptoms, the restrictive nature of the CME diet or due to feeding practices adapted by the parent and child. The number of allergic symptoms was the factor that was most strongly correlated with all three variables, however type of symptoms was also important. These three variables were in turn inversely correlated to dietary variety, meaning infants consuming a CME diet have a lower dietary variety than infants consuming a normal diet, with differing intakes of

particular food categories, specifically readymade baby food and sweet/miscellaneous foods. However, it should be emphasised that the feeding difficulties, fussy eating and food neophobia scores across the whole group were within normal ranges. There was no effect seen on growth overall, although there was an effect seen on weight status within the CME group according to SPT status. The four different groups of infants categorised by PCA (“milk-free”, “problem eaters”, “healthy” and “toddler” illustrated nicely that eating habits of infants and toddlers are multifactorial with type of diet, family history, breastfeeding status and age having differences on some, but not all traits.

5 Chapter Five: Study two

5.1 Overview

This chapter examines the eating habits of a group of school aged children who consumed a cows' milk exclusion (CME) diet in infancy, compared to a control group of children who consumed an unrestricted diet during infancy. Children and parents completed a number of standardised questionnaires; specifically measuring food preferences, fussy eating and food neophobia. Children undertook a simple test to measure preferences for the five basic tastes. Participants completed a four-day food diary to assess nutritional intake and basic growth measurements were undertaken. The results are discussed in relation to the potential long-term effects of following an exclusion diet during infancy on later eating habits.

5.2 Background

5.2.1 Rationale

To date, no research has evaluated if there is a long-term impact of avoiding cows' milk in early infancy on eating habits, growth and dietary intake. This study will address those issues and thus aim to make an original contribution to knowledge. The previous chapter demonstrated that infants who are consuming a CME diet have some differences in eating behaviour compared to those consuming an unrestricted diet (Maslin, Dean, Arshad, & Venter, 2015). Whether these differences are persistent over time is not known.

As previously outlined, CMA affects nearly 3% of young children in the UK. In the majority of children, CMA will resolve by age two years, when cows' milk products can successfully be tolerated (Schoemaker et al., 2015;Venter et al., 2008), although severe phenotypes exist with persisting CMA into older childhood (Saarinen et al., 2005; Skripak et al., 2007). The usual natural history of CMA therefore provides a unique opportunity to explore the effect of dietary exclusion in infancy on later dietary outcomes. This is in contrast to the exclusion of other food allergens, such as egg, fish or peanut, which would not easily lend themselves to such a study design. Although egg allergy has a similar natural history to CMA (Peters et al., 2014), with tolerance usually developing slightly later, cows' milk is unique in the fact that it predominates the early nutrition needs of infants, whether via infant formula or transmitted from the maternal diet via breast milk. Breast or formula milk is the sole source of nutrition in the first few months of life and remain the major source of nutrition for some time after the introduction of solid food. The exclusion of cows' milk therefore has arguably more

impact on nutrition and eating habits than the exclusion of other foods. It would also not be possible to implement this type of study design in children with other food allergens such as peanut or tree nuts as they tend to have a later age of onset and are generally more persistent into adolescence and adulthood (Fleischer, 2007).

There are several factors that contribute to the argument that consuming a CME diet in infancy may affect eating behaviour in later life. As previously described, taste preferences in early infancy are malleable and the altered taste of substitute formula has been shown to affect preference for savoury, sour and bitter foods in infancy (Mennella & Beauchamp, 2002) and up to the age of 4-5 years of age (Liem & Mennella, 2002). Secondly, we have demonstrated in the previous chapter that some symptoms caused by food allergens are associated with negative eating behaviours (Maslin et al., 2015), as have other authors (Meyer, Rommel, et al., 2014; Pentiuk et al., 2007). Whether the behaviours persist once the allergy is outgrown and the symptoms resolve is unknown. Finally, it is known that a proportion of food allergic children never introduce the culprit food allergen into their diet following a negative oral food challenge, possibly due to anxiety (Eigenmann, Caubet, & Zamora, 2006; Kim et al., 2011). This has potential to influence dietary intake if the food/food group is ubiquitous and nutrient dense, yet is rarely or never consumed. The effect of these factors on eating behaviour, growth and nutritional intake is unclear and considered together they provide a strong rationale for this study.

Irrespective of whether this study finds a positive, negative or neutral effect of consuming a CME diet in infancy on later eating habits, it will inform and raise awareness amongst health professionals. If a negative effect is reported, this provides more evidence and support for ensuring that fussy eating and feeding difficulties are better managed and food allergens are adequately reintroduced once the allergy has been outgrown. If a neutral or positive affect is found, this provides reassurance to health professionals and families alike that consuming a CME diet in infancy does not have a deleterious long term effect on childrens' eating behaviour, therefore it should be correctly adhered to for as long as is necessary.

5.2.2 Aim and objectives

The overall aim of this study was to determine if following a CME diet during infancy affects eating habits in later childhood, once cows' milk has been reintroduced into the diet.

The primary objectives of the study were:

1. To determine food preferences in children who consumed a CME diet during infancy compared to a control group who consumed an unrestricted diet during infancy.

2. To determine the degree of fussy eating and food neophobia in children who consumed a CME diet in infancy compared to a control group who consumed an unrestricted diet during infancy.

The secondary/exploratory objectives of the study were:

1. To determine taste preferences in children who consumed a CME diet during infancy compared to a control group who consumed an unrestricted diet during infancy.
2. To determine nutrient intake in children who consumed a CME diet during infancy compared to a control group who consumed an unrestricted diet during infancy.
3. To determine the growth status of children who consumed a CME diet during infancy compared to a control group who consumed an unrestricted diet during infancy.

These three objectives were set as secondary objectives as it was not realistic to recruit a sufficiently powered sample size within the allocated resources and timeframe to evaluate the effect of a CME diet on growth and nutritional intake or undertake an in depth investigation of taste preferences.

5.3 Justification for choice of questionnaires

An extensive literature search indicated that no one tool is suitable for assessing all aspects of childrens' eating habits (see appendix 8). Therefore, two validated parental report questionnaire measures were selected and used (appendix 9 and 11), in addition to one validated questionnaire completed by the child (appendix 19) and one other questionnaire that was constructed for the purpose of this study (appendix 20). Overall, questionnaires were chosen on the basis that they had previously been validated in the age group in question and could be completed in a reasonable timeframe. The following section provides detailed descriptions of these questionnaires and justification for why they were used.

5.3.1 Fussy eating

Fussy eating was measured using the Child Eating Behaviour Questionnaire (CEBQ) (appendix 9) (Wardle, Guthrie, et al., 2001). It is a parent-report measure designed to capture individual differences in children's eating style. It consists of four subscales with a total of 23 questions. All items are measured using a five point Likert scale, anchored from "never" to "always". Four questions are reverse scored. Subscale scores are calculated by taking the mean of the item ratings; higher scores reflect more of the behavior in question.

The original questionnaire was developed to identify individual differences in several

aspects of eating style that have been proposed to contribute to both underweight and overweight. The original questionnaire had eight subscales with 35 questions ("responsiveness to food", "enjoyment of food", "satiety responsiveness", "slowness in eating", "fussiness", "emotional overeating", "emotional under eating" and "desire for drinks"), which were developed based on a review of the literature and qualitative research. The questionnaire has been demonstrated to have high internal validity and test–retest reliability in the original validation study of 2-7 year old children (n = 131) (Wardle, Guthrie, et al., 2001) . Carnell and Wardle (2007) subsequently validated three of the subscales (slowness in eating, food responsiveness and enjoyment of food) in 4-5 year old children (n= 145) against behavioural measures of food intake. A later study (Ashcroft, Semmler, Carnell, van Jaarsveld, & Wardle, 2008) examined continuity and change in CEBQ scores from ages 4 to 11 years in a sample of 322 twin children, showing significant correlations for all CEBQ subscales. Finally, in a comprehensive review paper, the CEBQ was deemed to be one of the few questionnaires of children's eating behaviour to achieve all validation criteria, with demonstrable internal consistency, reliability and construct validity (de Lauzon-Guillain et al., 2012). In the current study the Cronbach alpha coefficients for each subscale ranged from 0.833 to 0.903, demonstrating good internal consistency.

Four of the eight subscales of the CEBQ were used in this study, as they are the subscales most relevant to fussy eating. These are the same subscales used in the study of fussy eating as per the method of Blossfeld et al. (2007). The four subscales used in this study were "enjoyment of food" (four questions), "food responsiveness" (five questions), "slowness in eating" (eight questions) and "fussiness" (six questions). The "food responsiveness" and "enjoyment of food" subscales both measure a child's appetite for food. The "enjoyment" subscale aims to capture normal variation in general appetite (e.g. ''my child enjoys eating''). However the questions in the "food responsiveness" subscale are designed to detect levels of appetite which could be viewed as maladaptive; such as eating in the absence of hunger or eating when prompted by external cues (e.g. ''given the choice, my child would eat most of the time''). The "slowness in eating" subscale includes eight items assessing satiety responsiveness. This is the extent to which a child stops eating or chooses not to start eating based on their perceived fullness (e.g. "my child gets full before his/her meal is finished"). Finally, the "fussiness" subscale measures the extent to which a child eats or tries to eat a variety of foods (e.g. "my child enjoys tasting new food"). Three of these subscales combined together (slowness, fussiness and food responsiveness) form a distinct "fussy eater" profile and are associated with avoidant eating (Tharner et al., 2014).

5.3.1.1 Other questionnaires that address fussy eating

The Child Feeding Questionnaire has been used in children aged 8-11 years old, however it has just three questions that address fussy eating, with the majority of the questionnaire aimed at assessing obesity proneness (Birch et al., 2001). As explained in chapter three, other more simplistic methods such as asking a single question "is your child a picky eater?" have also been used in school aged children (Goh & Jacob, 2012; Mascola, Bryson, & Agras, 2010; Xue et al., 2015). Finally, the Stanford Eating Questionnaire has been used in school-aged children in both Germany and the USA (Jacobi et al., 2008), but does not appear to have been used in a UK population as yet.

5.3.2 Food neophobia

Food neophobia is defined as a reluctance to try new and unfamiliar foods. In this study Food neophobia was measured using the Child Food Neophobia Scale (CFNS) (Pliner, 1994) (appendix 11). The questionnaire has ten items, five of which are reversed scored and a seven point likert scale from "strongly agree" to "strongly disagree". The scores are summed to give a food neophobia score ranging from 10-70, with a higher score indicating a higher level of food neophobia.

As already indicated in chapter four, the original Food Neophobia Scale (FNS) was developed for use in adults by Pliner & Hobden in 1992 (Pliner & Hobden, 1992) and was found to successfully predict behavior in willingness to consume novel foods, with good test retest reliability and internal consistency. It was later adapted to be a parental-report questionnaire for 5-11 year old children in Canada (Pliner & Loewen, 1997; Pliner, 1994). Using this tool, it was shown that parental reports of children's food neophobia were related to children's willingness to try novel food in a laboratory context. It has also been used extensively in studies of children in the UK (Cooke, Wardle, & Gibson, 2003; Cooke, Carnell, & Wardle, 2006). In the current study the Cronbach alpha correlation was 0.921, indicating good internal consistency.

Rather than use the shortened four- or six-item questionnaire, it was decided to use the original full 10-item questionnaire, as this was also being used in chapter four of this PhD. Using the same questionnaire in both studies would allow direct comparison of scores in two cross sectional groups from the same geographical area: infant/toddlers who are currently consuming a CME diet and older children who previously consumed a CME diet.

5.3.2.1 Other food neophobia measures

Other measures of food neophobia exist, namely The Food Attitude Scale (Raudenbush et al., 1995); which has not been adapted for use in young children and the Food Situations

Questionnaire (FSQ) (Loewen & Pliner, 2000) which is a self-report measure, therefore requiring children to be able to read and write. Although the children in this part of the study have the ability to read and write and complete the FSQ, it was decided to use the CFNS as it was also being used in the first study of this PhD, thus enabling a consistent approach. In addition the FSQ was developed and validated in Canada and therefore the food examples used in the questions may not be familiar or culturally relevant to participants in the UK.

5.3.3 Food preference questionnaire

Participants' food preferences were assessed using a Food Preference Questionnaire developed by Cooke & Wardle (2005) (appendix 19). The questionnaire consisted of a list of 119 common food and drink items. The list included 'single' foods (e.g. ham, banana), 'composite' foods (e.g. lasagne, meat pie), 'condiments' (e.g. mayonnaise, ketchup) and drinks (e.g. fizzy drinks, semi-skimmed milk). Foods were divided into nine different categories for the purpose of data analysis (meat, processed meat, fish, eggs, fruit, vegetables, dairy, starchy staples and sweet/fatty). The original study reported Cronbach alpha coefficients of > 0.70 for all food categories of food, with exception of fish, demonstrating good internal consistency. For the current study, Cronbach alpha coefficients were > 0.70 for all categories except fish (0.546), processed meat (0.644) and starchy staples (0.673).

Children were asked to indicate 'how much you like each food by ticking the appropriate box'. There were six response alternatives – 'never tried it', 'I hate it', 'I don't like it', 'it's OK', 'I like it' and 'I love it'. Responses were scored from 1- 6 respectively. Children were advised to complete the questionnaire based on their own individual preferences. They were advised that there was no correct answer and to answer the questions based on what they actually liked, not what their parents thought they should eat or what they actually ate. Participants were encouraged to ask the researcher if they had any difficulty with the questionnaire or did not understand any of the food items. On average, the questionnaire took approximately 10-15 minutes to complete.

Cooke's questionnaire was based on a questionnaire developed by Wardle et al. (Wardle, Sanderson, et al., 2001) in a study of 4–5-year-old children in London. Wardle's original questionnaire had 94 food items, derived from FFQs from two large epidemiological studies. The list of food items was increased from 94 to 115 for Cooke's study to encompass a wider range of foods typically eaten by older school age children and adolescents. Wardle's questionnaire was completed by mothers as a parent-report questionnaire and was also used as a self-report measure in 9-11 year old children in a study by Gibson et al. (Gibson, Wardle,

& Watts, 1998).

In Cooke & Wardle's 2005 study, the sample size was 1291 school children aged 4-16 years. Parents completed the questionnaire on behalf of those aged 4-7 years old. Children and adolescents aged 8-16 years old completed the questionnaire independently under the supervision of a teacher. For this study, it was decided to ask participants directly regarding their own preferences rather than their parents, in order to reduce bias. In addition it was felt that children of this age would have the cognitive ability and understanding to independently complete the questionnaire. This was confirmed by the original author who reported that eight year old children did not have any problems completing the questionnaire independently (personal communication with Dr. Lucy Cooke 21st January 2013).

5.3.3.1 Food preference questionnaires from other countries

One of the reasons for selecting this particular questionnaire was because it was developed using food lists from the UK. Studies conducted in other countries have used similar questionnaires to measure food preferences, however with different methods of composing the food lists and different response options. Skinner et al.'s (Skinner, Carruth, Bounds, & Ziegler, 2002) study of longitudinal food preferences in the USA used a list of 196 food items. However they only had three response options: "like", "dislike" and "never tasted", which the authors acknowledge did not allow an in depth analysis of preference. An Australian study used a food preference questionnaire composed of 176 items (Russell & Worsley, 2008). Their food list was constructed from a combination of sources: a food variety index, unpublished data on children's dietary intake, supermarket lists and a national healthy eating guide. In a longitudinal French study, Nicklaus et al. (2004) used a questionnaire composed of the 80 foods frequently offered at lunch at the nursery canteen and used an 85mm unstructured scale anchored to determine responses. Overall these three questionnaires used a broadly similar approach, however it is significant that they each used country and culture specific food lists. As is the case with FFQs, it is important that the food list in preference questionnaires includes food items that are relevant to both the population and the research question.

Other studies have assessed food preference using brief questionnaires, which don't provide a detailed amount of data. For example Ton Nu et al. (1996) simply asked participants to list their ten most and least favourite foods. A Spanish study asked six questions about preferred foods (Pérez-Rodrigo, Ribas, Serra-Majem, & Aranceta, 2003). Other studies have assessed preference for a specific food category such as snack foods (Rollins et al., 2010). None of these approaches were felt to provide sufficient detail for this study.

5.3.3.2 Computerised food preference questionnaires

For the purpose of this study, as the objective was to determine food preferences to a range of foods, the food list needed to be comprehensive rather than only focus on one particular food group. Some recent studies have used computerised questionnaires, using images of foods rather than text. However these type of online questionnaires tended to only focus on one particular food group, often focusing on a narrow range of "healthy" or "unhealthy' foods. For example Rodenburg et al. (Rodenburg, Oenema, Pasma, Kremers, & van de Mheen, 2013) assessed both food and activity preferences in primary school children using a newly-developed computerised visual instrument, however the food preference included only four 'snack' items. Vereecken et al. (2010) also used a computerised assessment tool, but this only focused on fruit and vegetables.

5.3.3.3 Direct measurement of food preferences

Questionnaires are quick and simple to administer, allowing the assessment of preferences to a large number of foods in a short period of time. Analysis of a large number of different foods allows for description of patterns of food intake. However, it must be emphasised that questionnaires are indirect measurements of food preference. Direct measurement of food preferences requires tasting and consumption of food, usually in a laboratory setting. There is a wealth of studies describing and validating these methods in children of all ages (Birch, 1979; Léon, Couronne, Marcuz, & Köster, 1999; Liem & Zandstra, 2010) and it has been shown that using real food as a stimuli produces the most reliable measure, moreso than photographs or food models (Guthrie, Rapoport, & Wardle, 2000).

However a limitation of laboratory studies is that they require specific research facilities and only a limited number of foods can be tested at once. Due to the labour-intensive nature of these methods, they are usually only used to validate questionnaires or used commercially to evaluate specific aspects in the development of new food products (e.g. saltiness/creaminess). The use of sensory testing to measure taste preferences will be discussed in more detail later in this chapter.

5.3.4 Weaning history questionnaire

As the majority of participants were recruited from two prospective birth cohort studies, data on weaning, infant feeding habits and food allergy history during infancy was available from the original studies (Grimshaw et al., 2014; Venter et al., 2008). The birth cohort studies are explained later in this chapter. A questionnaire was constructed to collect relevant information on social demographics, family history of allergy, allergic history, infant feeding, relevant

medical history and growth history of participants from parents and the original study data set (see appendix 20). This enabled information on potential confounding variables to be documented.

The socio-demographic section of the questionnaire included questions on age of participant, gender, ethnic origin, maternal age, parental occupational status and parental educational level. The family history of allergy section asked whether either parent of the participant or any sibling had ever had symptoms of asthma, hayfever, eczema or food allergy. The allergy history section asked whether the participant had ever had symptoms of asthma, hayfever, eczema or food allergy. The food allergy section asked which foods had been excluded, at what age, why and when they had been introduced. These questions were adapted from the International Study of Asthma and Allergies in Childhood (ISAAC) (Asher et al., 1995) questionnaire. The infant feeding section asked about breastfeeding, use of different formula feeds, age of introduction of solid foods, type of weaning foods, whether any vitamins were taken and how much attention was paid to healthy eating (three point scale). Also included were questions about birth weight and whether the child had any other medical conditions.

A small proportion of participants (n = 5) were not recruited from the two birth cohort studies. They were recruited from retrospective NHS records. For these participants, all the above questions were collected from the parents and cross-checked against medical and dietetic records.

5.3.5 Nutritional Intake

Parents and children were asked to jointly complete a four day estimated food diary (National Diet and Nutrition Survey food diary (see appendix 21). The diary was adapted slightly to make it easier to complete. Participants were encouraged to keep the diary for four consecutive days, including one weekend day. Clear instructions of how to complete the food diary were given orally and in writing. The instructions included advice on estimating portion sizes using household measures, a reminder to include information on cooking method, brand names, wastage, snacks and extras consumed both at home and when away from home. A section was provided for details of recipes and ingredients used. After each day parents were asked to rate whether it was a typical day's food consumption for their child (i.e. if they ate and drank more or less than usual).

Parents were provided with a stamped addressed envelope and asked to return the diary. If the food diary was completed in insufficient detail, a phone call or email was made to the parent to clarify the details (e.g. portion size offered). If the food diary had not been returned

within one month, a phone call or email was made to prompt the parents to complete it and send back.

5.3.5.1 Strengths and limitations of using a food diary as a dietary assessment method

5.3.5.1.1 Strengths of food diary as a dietary assessment method

As previously discussed in chapter four, there is no perfect method of dietary assessment, with all methods having inherent limitations. There is also no universal criteria for choosing a dietary assessment method in children (Livingstone & Robson, 2000). An adapted version of the food diary from the UK National Diet and Nutrition Survey (NDNS) (2008/2009 and 2010/2011) was used in this study. A four-day estimated food diary was chosen for several reasons. Firstly, a food diary allows a more detailed description and quantification of food consumed compared to other methods (e.g. brand of food, cooking method, portion size offered). Use of an estimated food diary rather than a weighed food diary means less participant burden. Indeed it is thought that the novelty and curiosity of assisting with food diary recording in 7-12 year olds may help to maintain enthusiasm and compliance in food record completion (Livingstone, Robson & Wallace, 2004), making it a suitable method for this age group. A recent European study also found that a food diary method was viewed as feasible and understandable for collecting dietary data in children with only a minority of respondents finding food description and quantification difficult (Ocke et al., 2015).

The diversity of the diet, individual variation in food intake and within individual variation in food intake will affect the number of days needed to measure energy and nutrient intake in children. Five days was found to be appropriate in young children (Lanigan, Wells, Lawson, Cole, & Lucas, 2004). Four days of recording is used by the NDNS. It is a reasonable timeframe to collect data on most nutrients, without the demands of completing a diary for a full week, with the exception of some trace micronutrients that require an extended period for accurate intake recording. It is also known that the quality of dietary reporting deceases during the reporting period due to fatigue (Livingstone & Robson, 2000). Indeed the NDNS previously used a seven-day food diary, but reduced it to four days in order to reduce participant burden. A 24-hour dietary recall would have been an alternative dietary assessment option, however it would only have provided a very short-term reflection of dietary intake and therefore less data to analyse. Finally, an estimated food diary has been the method used by other studies assessing food allergy in children (Berry et al., 2015; Christie, Hine, Parker, & Burks, 2002;

Flammarion et al., 2011; Meyer et al., 2014; Tiainen, 1995; Tuokkola et al., 2010), therefore will allow direct comparison of results with the existing literature.

5.3.5.1.2 Limitations of a food diary as a dietary assessment method

There are some limitations of using an estimated food diary to assess nutritional intake. Firstly, the method was not validated against any biomarkers in this particular age group, as this was not feasible. However the use of a food diary from a well-established national cohort study was deemed to be a robust and valid method. Due to a food diary taking a number of days to complete, rather than a 24-hour recall or FFQ which can be completed immediately, there is a level of responsibility for the participant to prospectively complete and return the diary. This has potential to lead to response attrition and non-response bias as some participants will not be willing to or forget to complete or return the food diary. Reminder phone calls were made to overcome this problem. The process of completing a food diary is known to lead to a subconscious change in eating habits, known as the "Hawthorne effect". Participants may omit certain snacks and underreport food that they have eaten, particularly if they are considered unhealthy or unsuitable, known as social desirability bias (Moore, Tapper, Moore, & Murphy, 2008). To address this, parents and children were encouraged to record all food and drink intake accurately and honestly and reassured that the food diary was not a "test" of healthy eating.

The fact that the participants were school-aged children who spend a large proportion of weekdays away from parents, may mean that parents are not accurate reporters of daytime food intake (Black & Livingstone, 2000). To overcome this potential problem, children were encouraged to be actively involved in helping with completion of the food diary and local school lunch menus were consulted if there was any discrepancy. From the age of 7-8 years old, it is thought that children are aware of their food intake and can begin to conceptualize time, however it is not until about 12 years old that they have sufficient recall and estimation skills to accurately report food without parental assistance (Livingstone et al., 2004). Therefore, this approach was deemed to be suitable and most practical for the age group recruited. Finally, the coding and analysis of food diaries is particularly time consuming for the researcher and can be subject to error. To overcome this limitation, a standardised proforma was used to ensure that food diaries were coded consistently.

5.3.6 Taste preference

Taste preference was assessed for the five main tastes: sweet, salty, bitter, savoury (umami) and sour based on the methodology of Knof et al. (2011) and Liem & Mennella (2002).

Participants were asked to taste and rate five different flavoured waters using a child-orientated rating scale (Popper & Kroll, 2005) (appendix 22). A sixth sample consisted of plain water.

5.3.6.1 Procedure

Samples were prepared in advance using bottled water and kept refridgerated until immediately before the test. Substrates were weighed using a calibrated laboratory weighing scales exact to 0.001gram. The dilution of each substrate is shown in Table 5.1. Samples were identical in appearance.

Taste preference tests took place in a well-ventilated room. Samples were presented individually in opaque cups in a counterbalanced order. After tasting each sample, each child was asked to rate it on a child-orientated verbal scale, consisting of nine ratings from "superbad" to "supergood" (see appendix 22) (Popper & Kroll, 2005). After a rating was made, the child was allowed to drink some plain water, before continuing to the next sample. Parents were asked to sit quietly, so that they did not influence their child's reaction. The whole process took approximately 5-10 minutes. All measurements were conducted and supervised by the same researcher to minimise measurement error.

Table 5.1 Concentration of taste solutions

Taste Modality	Ingredient	Dilution (mmol/L)
Sweet	Sucrose	46.7
Salty	Sodium Chloride	27.4
Bitter	Caffeine	1.3
Umami (savoury)	Monosodium glutamate	9.5
Sour	Citric acid	40

5.3.6.2 Justification for choice of substrate and doses

The substrates used were based on that of Knof et al. (2011) and Liem & Mennella (2002). Knof et al.'s (2011) European study of 191 children indicated that the majority of children can detect these tastants at this level of solution for sucrose (sweet), sodium chloride (salty), caffeine (bitter) and monosodium glutamate (umami/savoury). The level of citric acid (sour) was chosen based on the study protocol by Liem & Mennella (2002).

As the measurement of taste preference was an *exploratory* objective, all five tastes were assessed in this study, rather than focus on one or two specific tastes. Although previous studies of children fed substitute formulas for CMA have predominantly focused on sour and bitter tastes (Liem & Mennella, 2002; Mennella & Beauchamp, 2002; Mennella, Forestell,

Morgan, & Beauchamp, 2009), it was decided to include all five tastes so that each could be correlated against other outcome variables (e.g. food preference, growth) in a speculative manner. Previous studies (Liem & Mennella, 2002) have measured preference using several different concentrations of each solution, with preference testing occurring on different days. Because it was necessary to keep the procedure relatively quick and simple, a basic procedure with only one concentration of each taste solution was used. Other studies have prepared the tastants using flavoured juice/punch, or foods such as crackers or cereal (Kimmel, 1994; Knof et al., 2011; Liem & Mennella, 2002; Mennella & Beauchamp, 2002), however water was used as the solution in order to minimise the influence of colour, appearance or other flavours or textures. A previous sensory study in 8-16 year old children using different flavoured substitute milks have shown that a chocolate flavour can override all other sensory properties, thus distracting from the evaluation (Palacios et al., 2010). Water has been successfully used as a solution for tastants in previous studies in young children (Schwartz, Issanchou, & Nicklaus, 2009).

5.3.6.3 Choice of measurement scale

Studies of taste preference in younger children (aged 3-5 years old) generally employ a simple paired preference or ranked order test to determine taste preference. However, school aged children are thought to have sufficient language, memory and reasoning skills to use a hedonic scale (Popper & Kroll, 2005), which is more discriminating and provides more information that a basic forced choice paired preference test (Kimmel, 1994; Popper & Kroll, 2005).

Previous research has indicated that children aged 4-10 years old can reliably and consistently express preferences with a hedonic scale (Léon et al., 1999). Unlike children aged 5-7 years old who may perform better using a pictorial scale, children aged 8-10 years old are deemed to be “semi literate”, with the ability to read at some level, but may not understand certain words such as “moderately” or “extremely” (Kroll, 1990), hence the development of a child friendly scale using words such as “really bad”. A nine-point scale was deemed to be as good as, if not better than a seven-point scale as it provided better discrimination (Kroll, 1990), therefore the Popper and Kroll nine point hedonic scale was an ideal choice for this study.

5.3.7 Growth

Weight was measured using electronic scales in kg to one decimal place. Height was measured using a stadiometer in cm to one decimal place. Participants were asked to remove outer clothing (i.e. coats and jackets) and shoes for these measurements. Height and weight were plotted on a standard UK growth chart. Body Mass Index (BMI) was calculated using the

standard calculation: weight in kg/(height in metres)^2. Weight for age percentile was calculated manually using a UK growth chart, by dividing the actual weight by the 50th percentile weight. Height for age percentile and BMI percentile were calculated and converted into Z scores using the World Health Organisation (WHO) Anthro Plus software (WHO, 2007).

Waist circumference was measured in cm to the nearest decimal place and plotted on a UK waist circumference centile chart. It was measured as the “narrowest waist”, which is the most frequently recommended site and is easy to identify (Wang et al., 2003). Waist circumference was measured as an indicator of fat mass as recent research has indicated that obesity may be just as prevalent, if not more of a concern, as underweight in children consuming exclusion diets (Meyer et al., 2014). Waist circumference is proposed to be a better measurement of excess abdominal fatness, than BMI alone which is a measure of excess general fatness, but does not give an indication of body fat distribution (McCarthy, 2006). All measurements were conducted by the same researcher using the same equipment.

5.4 Method

5.4.1 Study design

This was a cross sectional study of 7-13 year old children from the Isle of Wight and the Winchester area in the county of Hampshire, UK. Figure 5.1 summarises the study design. Participants were predominantly recruited from two birth cohort research studies, the FAIR and PIFA studies. The FAIR study is the Food Allergy and Intolerance Research study, which investigated the prevalence of food allergies and intolerances in children born on the Isle of Wight between 2001/2002 (Venter et al., 2008; Venter et al., 2009). The PIFA (Prevalence of Infant Food Allergy) study which recruited children born in 2006/2008 from the Winchester area, was part of the Europrevall study, funded to look at the prevalence of food allergies in Europe (Grimshaw et al., 2013, 2014). For both of these studies, detailed prospective information was obtained about feeding practices in infancy.

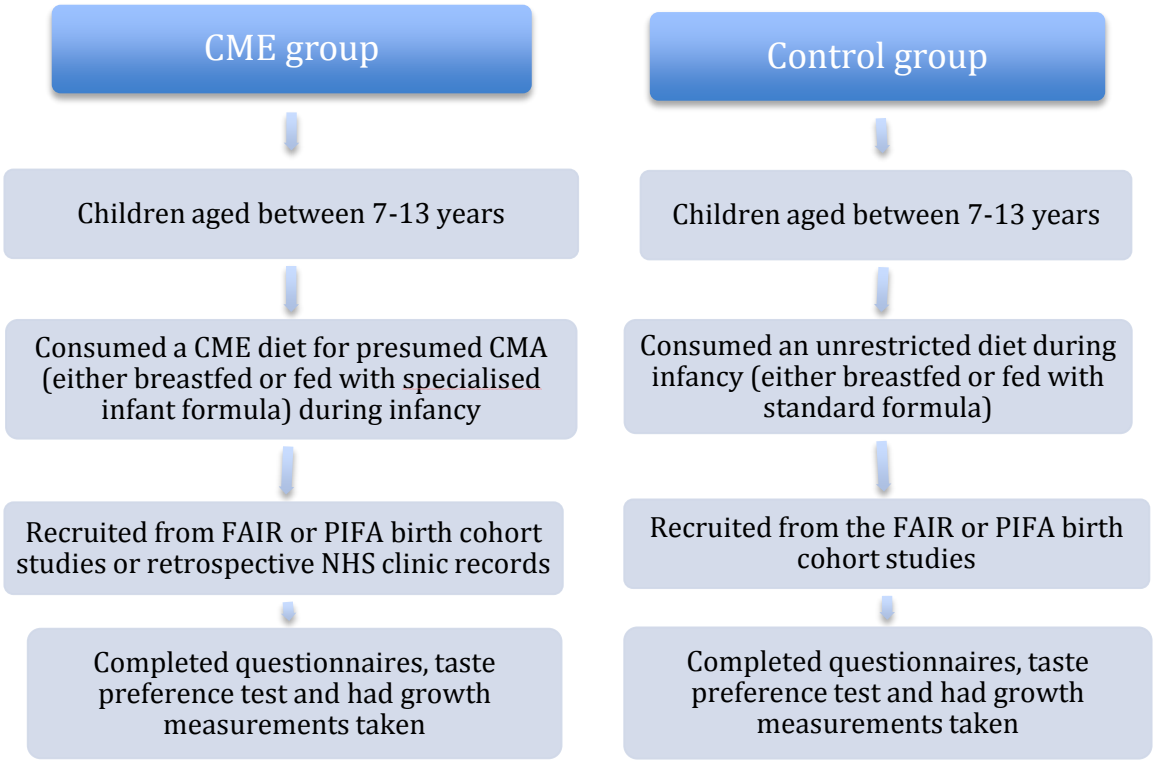

Figure 5.1 Summary of study design

5.4.1 Sample

5.4.1.1 Identification of participants

This study had two groups: an experimental group and a control group. The experimental group was composed of children who had consumed a CME diet during infancy for the treatment of documented or likely CMA. Children were eligible for the CME group if they had consumed a substitute formula and/or a cows' milk free diet in the first year of life for a period of three months or longer. Children who were excluding other foods (i.e. egg or soya), in addition to cows' milk were eligible to take part. These children were recruited from the FAIR and PIFA studies, with a small number of participants recruited from NHS allergy clinic records.

The control group was composed of children who had consumed an unrestricted diet during infancy. All participants in the control group were recruited from the FAIR and PIFA studies. Children with any additional medical conditions requiring a special diet (e.g. Type 1 diabetes, cystic fibrosis) were excluded from the study. Children with current food allergy were excluded from the study.

5.4.1.2 Sample size

Sample size calculation was discussed and confirmed by the statistician at the University of Portsmouth School of Health Sciences and Social Work. All sample sizes were calculated using Gpower 3 for a two-tailed outcome, at 80% power and at a significance level of 0.05. Sample sizes were calculated for primary objectives only. Sample size calculations were made on the basis of a ratio of 1:2 CME: control group, as it was known that there would be a much greater number of control participants eligible for recruitment than CME participants.

5.4.1.2.1 Food preferences

According to previous research of food preferences in 8-11 year old children (Cooke & Wardle, 2005), children liked 60 (+/- 17) of foods studied. Assuming the previously milk-free children will like +/- 15% more foods than the control group, 43 children and required in the experimental group and 86 in the control group To detect a 15% difference in liking scores for the individual food groups of fruit, vegetables, processed meat, meat, dairy, starchy foods and fatty & sugary foods, requires 43 children and required in the experimental group and 86 in the control group

5.4.1.2.2 Fussy eating

Previous research of 11-year old children in London (Ashcroft et al., 2008) indicates mean scores of between 2.5-3.7 (SD 0.8) for the 4 different subscales of the CEBQ. Assuming there will be a difference of 0.4 of a mean score between the milk free and control groups, requires 48 children in the experimental group and 96 children in the control group.

5.4.1.2.3 Food Neophobia

Research on neophobia levels in 11-12 year old children (Pliner & Loewen, 1997) reports a mean score of 31 (SD 10) for boys and 40.90 (SD 14) for girls. Assuming a difference of 7 between groups, requires 37 children in the CME group and 74 in the control group.

In summary, to ensure the study was sufficiently powered for all of the primary objectives, required 48 participants in the CME group and 96 participants in the control group.

5.4.2 Ethical considerations

Ethical approval for the study was obtained from Berkshire NHS ethics committee in May 2013 (see appendix 15). Local approval was obtained from the Isle of Wight NHS Trust Research and Development Committee in July 2013 and University Hospital Southampton NHS Foundation Trust in July 2014 (see appendices 14 and 23). Parents and children were provided with a study information sheet each (see appendices 23, 25 and 28). Written informed consent

was obtained from both parent and child (see appendices 26 and 27). Parents and children were free to withdraw from the study at any time. Data was anonymised and when not in use, secured in locked cabinets or password protected in the case of electronic records. Online questionnaires were administered via Bristol online surveys (http://www.survey.bris.ac.uk), for which there is encryption, ensuring the data could not be intercepted by third parties. Participants were given a £5 voucher as a thank you for their time.

5.4.3 Recruitment

Recruitment took place between July 2013 and April 2015. Participants eligible for inclusion in the CME group were identified by the study coordinators for the FAIR and PIFA studies from the study databases. Control participants were identified as the four consecutive study participants to each identified CME participant in the database (i.e. if ID number 6 was identified as a potential CME participant, ID numbers 7,8, 9 and 10 were identified as potential control participants). All identified participants were posted a study information pack, including parent and child consent forms. Parents who were interested in the study and returned the consent forms were telephoned and an appointment was arranged. Parents who were interested in taking part in the study but were unwilling to attend an appointment were given the option to complete the questionnaire online or by post.

A small number of potential participants for the experimental group were identified from NHS clinic records by the researcher. These parents were recruited in the same way (see appendix 28).

5.4.4 Administration of questionnaires

Questionnaires were mostly self-administered, however the researcher was available to clarify any queries and double-check any omissions. Questionnaires to be completed by children were clearly explained in age-appropriate language.

5.4.5.1 Questionnaire coding

5.4.5 Data analysis

Questionnaires were scored and coded according to published guidelines by the original authors. Where the scoring of the questionnaires was not published, the author was contacted for this information. Median values were calculated for the individual subscales of each questionnaire. A coding logbook was maintained to ensure consistency in coding of questionnaires.

5.4.5.2 Food diary coding

Food diaries were coded by the PhD student (KM), who is an experienced dietitian, using a predetermined protocol. The protocol for coding the food diaries was as follows. Portion sizes were estimated using published age-appropriate portion sizes (Patel, Vyas, Custovic, & Murray, 2012; Wrieden et al., 2008). If portions were not available from these two sources, portions sizes were estimated using the Food Standards Agency's reference book on food portion sizes (Food Standards Agency 2002). Information on portion sizes of popular take away foods (e.g. Mc. Donalds) and supermarket foods was obtained from the manufacturers' websites. Composite items such as sandwiches or recipes for composite dishes were analysed by diving the item into separate components or ingredients. Standard foods were used if foods recorded lacked sufficient detail (e.g. type of cheese was entered as cheddar). Missing portion sizes were estimated by weight of food consumed on other days. Twenty percent of food diaries were verified by a second dietitian.

5.4.5.3 Food diary analysis

Food diaries were analysed using nutritional analysis software Dietplan 6 (Forestfield Software Limited, Horsham, UK). Information on foods not included in the database and dietary supplements were obtained from the manufacturers' websites and added to the software. Intake was compared to Estimated Average Requirements (EAR) and Recommended Nutrients Intakes (RNI) for macro and micronutrients (Dept. of Health, 1991).

5.4.5.4 Data checking

Ten per cent of files were double entered and compared to test consistency and minimise errors of data entry.

5.4.5.5 Statistical analyses

Data was analysed using SPSS software (IBM, version 20). Missing values were computed as "999". All data sets were double checked for outliers. All continuous variables were tested for normality of distribution using a one sample Kolmogorov-Smirnov test to determine normality. Descriptive statistics were calculated for all variables. Categorical variables were expressed as numbers and frequencies. Continuous variables were expressed as median/mean and standard deviation/minimum and maximum.

As most of the variables were non-parametric, differences between the CME and control groups were compared using Mann Whitney tests for all primary outcome variables. For categorical variables, the X^2 test was used. Analysis of Covariance (ANCOVA) and two way Analysis of Variance (ANOVA) tests were undertaken to compare groups, whilst controlling for

covariates (e.g. age, gender, family history of food allergy).Spearmann rho correlations were performed to identify any relation between the main outcome variables. The significance level was set at 0.05 for all analyses.

5.5 Results

5.5.1 Description of sample

5.5.1.1 Demographic statistics

In total, 101 participants were recruited, 28 in the CME group and 73 in the control group. The number of participants recruited from the FAIR and PIFA studies and NHS records is shown in Figure 5.2.

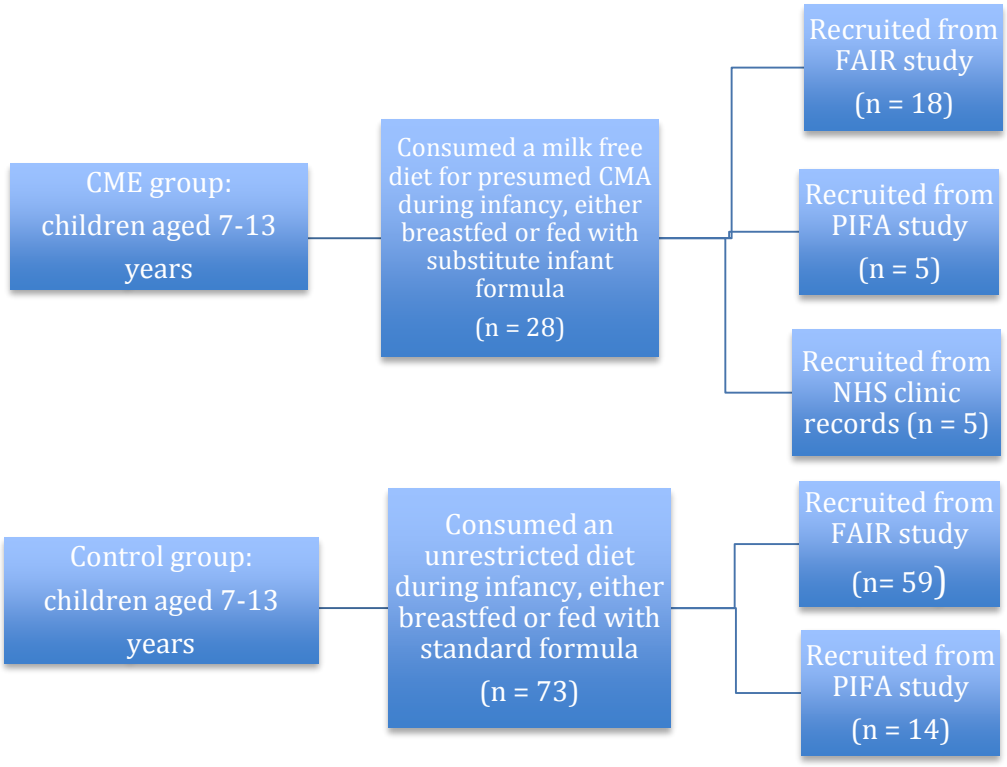

Figure 5.2 Source of participant recruitment into the study

Demographic characteristics of participants are detailed in Table 5.2. There were proportionately more girls in the CME group than the control group (57.1% compared to 43.8%), but this difference was not statistically significant. The median age of all participants was 11.5 years. Six participants (8.3%) were born preterm (< 37 weeks), with a median gestation of 35.5 weeks (range 34-36 weeks). Three sets of twins were recruited, all from the FAIR study. The parents of participants were well educated, with more than a third of parents having a graduate or postgraduate qualification.

No significant difference was found between the CME and control groups for age, ethnicity, number of siblings, parental education or occupation, birth weight, gestation or paternal food allergy history. Significant differences were found for: maternal and sibling food allergy history (chi square $p = 0.036$ and $p = 0.021$ respectively), with those in the CME group having higher rates of both. The majority of questionnaires were completed by mothers (92%); with fathers completing 5%, guardians 2% and grandparents 1%.

Table 5.2 Demographic characteristics of participants

	All (*N* =101)	CME group (*n* =28)	Control group (*n* = 73)
Age in years (median)	11.5	11.33	11.58
(minimum-maximum)	(7.04 – 13.83)	(7.25 – 13.83)	(7.04 – 12.44)
Female (%)	48 (47.5)	16 (57.1)	32 (43.8)
Male (%)	53 (52.5)	12 (42.9)	41 (56.2)
Number of siblings	1 (0-5)	1 (0-4)	1 (0.5)
Ethnicity			
White British (%)	98 (97)	28 (100)	70 (95.9)
Maternal age (median)	42.5 (29-53)	43 (32-51)	42 (29-53)
Maternal occupation			
Student (%)	2 (2.0)	1 (3.6)	1 (1.4)
Self-employed (%)	13 (13.0)	4 (14.3)	9 (12.5)
Full time (%)	32 (32.0)	6 (21.4)	26 (36.1)
Part time (%)	36 (36.0)	14 (50.0)	22 (30.6)
Unemployed (%)	6 (6.0)	1 (3.6)	5 (6.9)
Other (%)	11 (11.0)	2 (7.1)	9 (12.5)
Paternal occupation			
Student (%)	0 (0.0)	0 (0.0)	0 (0.0)
Self employed (%)	20 (20.8)	7 (25.9)	13 (18.8)
Full time (%)	70 (73.0)	19 (70.4)	51 (73.9)
Part time (%)	3 (3.1)	0 (0.0)	3 (4.3)
Unemployed (%)	3 (3.1)	1 (3.7)	2 (2.9)
Other (%)	0 (0.0)	0 (0.0)	0 (0.0)
Maternal education			
None (%)	2 (2.0)	0 (0.0)	2 (2.7)
GCSE /A-level or equivalent (%)	62 (62.0)	20 (74.0)	42 (57.5)
Graduate / Postgraduate (%)	36 (36.0)	7 (25.9)	29 (39.8)
Paternal education			
None (%)	8 (8.1)	3 (11.1)	5 (6.9)
GCSE /A-level or equivalent (%)	56 (56.6)	17 (62.9)	39 (54.1)
Graduate / Postgraduate (%)	35 (35.3)	7 (25.9)	28 (38.9)
Family history of food allergy			
Maternal (%)*	23 (22.5)	10 (35.7)*	13 (17.8)*
Paternal (%)	16 (15.6)	7 (25.9)	9 (12.3)
Sibling (%)*	18 (17.6)	10 (35.7)*	8 (11.0)*

*Difference between CME and control group significant $p < 0.05$ using a chi square test

5.5.1.2 Infant feeding characteristics of sample

Details of participants' infant feeding history are shown in Table 5.3. This information was collected prospectively for all participants from the original birth cohort studies, with the exception of the five participants recruited from the NHS allergy clinic records, for who the answers were based on recall. The questions regarding "age of introduction of lumpy and finger foods" and the "predominant type of weaning food" were not collected in the birth cohort studies,

so were based on recall for all participants. Infant feeding data was available for all but one participant.

The majority of participants had been breastfed at one stage (78.0%) (i.e. ever been breastfed). However significantly more of the control group participants had ever been breastfed compared to the CME group (chi square p = 0.02) and they were breastfed for longer (chi square p = 0.017). All of the CME group (100%) had been given formula milk, compared to 87.5% of the control group. No significant difference was found between groups for ever been fed formula milk or age at introduction of formula milk.

The median age of solid food introduction was 16 weeks in both groups. The most common first baby food was baby rice (77.7%), which was the same for both groups. The predominant type of baby food used differed between groups (chi square p = 0.018), with a greater proportion of those in the CME group using readymade baby food (14.3% compared to 1.4% of the control group).

Table 5.3 Infant feeding history of participants

	All (*N* = 100)	CME group (*n* = 28)	Control group (*n* = 72)
Ever breastfed			
Yes (%)*	78 (78.0)	17 (60.7)*	61 (84.7)*
No (%)	22 (22.0)	11 (39.3)	11 (15.3)
Ever given formula milk			
Yes (%)	91 (91.0)	28 (100.0)	63 (87.5)
No (%)	9 (9.0)	0 (0.0)	9 (12.5)
*Breastfeeding duration**			
Never (%)	22 (22.0)	11 (39.4)*	11 (15.3)*
< 1 month (%)	16 (16.0)	2 (7.1)*	14 (19.4)*
1-3 months (%)	17 (17.0)	6 (21.4)*	11 (15.2)*
3-6 months (%)	12 (12.0)	6 (21.4)*	6 (8.4)*
6-9 months (%)	10 (10.0)	1 (3.6)*	9 (12.5)*
9-12 months (%)	14 (14.0)	2 (7.1)*	12 (16.7)*
> 12 months (%)	9 (9.0)	0 (0.0)*	9 (12.5)*
Age at introduction of formula milk			
Never (%)	9 (9.0)	0 (0.0)	9 (12.5)
< 1 month (%)	52 (52.0)	16 (57.1)	36 (50.0)
1-3 months (%)	15 (15.0)	5 (17.9)	10 (13.9)
3-6 months (%)	16 (16.0)	4 (14.3)	12 (16.7)
6-9 months (%)	6 (6.0)	1 (3.6)	5 (6.9)
9-12 months (%)	2 (2.0)	2 (7.1)	0 (0.0)
First weaning food			
Baby rice	74 (77.7)	20 (76.8)	54 (78.4)
Fruit	5 (5.3)	0 (0.0)	5 (7.2)
Sweet potato/carrot/parsnip	5 (5.3)	1 (3.9)	4 (5.8)
Broccoli/green vegetable	1 (1.1)	1 (3.9)	0 (0.0)
Rusk	2 (2.1)	1 (3.9)	1 (1.4)
Porridge/oats	5 (5.3)	3 (11.5)	2 (2.9)
Other	3 (3.2)	0 (0.0)	3 (4.3)
Age at introduction of solid foods (weeks)	16 (10-26)	16 (11-24)	16 (10-26)
Age at introduction of lumpy foods (weeks)	24 (14 – 208)	24 (16-208)	24 (14-52)
Age at introduction of finger foods (weeks)	26 (16 – 222)	24 (16-96)	27 (16-222)
Predominant type of weaning food			
Homemade (%)	50 (50.5)	15 (53.6)	35 (49.3)
Readymade baby food (%)*	5 (5.1)	4 (14.3)*	1 (1.4)*
A mixture of both (%)	44 (44.4)	9 (32.1)	35 (49.3)

*Significant difference between CME and control groups (chi square p = < 0.05)

5.5.1.3 Dietary exclusion

Within the CME group, 14 participants (50%) had a history of excluding cows' milk only. The number and type of foods excluded by the CME group is displayed in Figure 5.3. The highest number of foods excluded by a participant was three. Eleven participants (39.3%) were excluding two foods and three participants (10.7%) were excluding three foods. The median age of exclusion for cows' milk was 10.5 weeks (range 1-36 weeks).

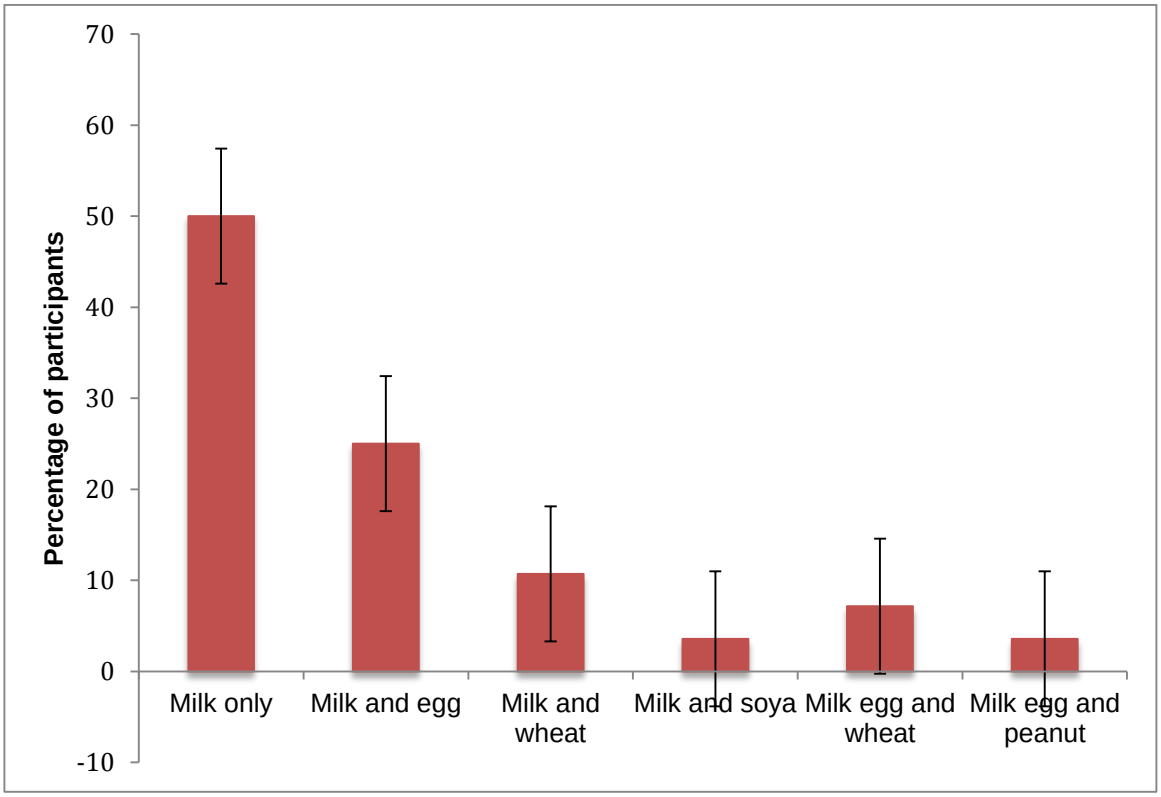

Figure 5.3 Foods excluded by the CME group

5.5.1.3.1 Use of substitute formula for CMA

All participants in the CME group used a substitute formula at some stage. Substitute formula was initiated at a median age of 11.5 (range 2-40 weeks), with a median duration of substitute formula usage of 67.5 weeks (range 16-205 weeks i.e. four months to four years). The type of substitute formula used is shown in Figure 5.4. The most commonly used formula was soya, used by 50% of the CME participants. Four participants (14.3% of CME group) were given a second category of substitute formula due to not tolerating the first: two participants changed from soya to EHF whey, one participant changed from EHF whey to soya and one participant changed from EHF casein to EHF whey.

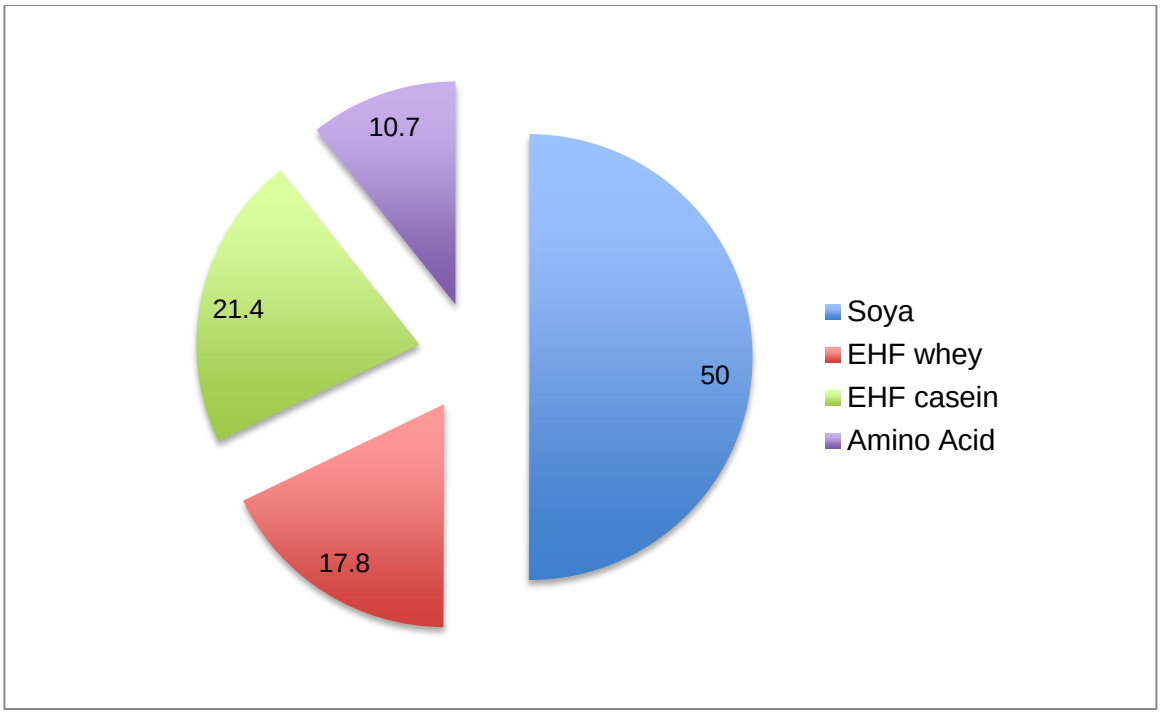

Figure 5.4 Type of substitute formula used by the CME group (%)

5.5.1.4 Skin prick test status

Four participants (14.3% of CME group) had a positive SPT to cows' milk; 24 (85.7%) had a negative SPT to cows' milk. There was no significant differences found between the SPT positive and SPT negative groups for any growth measurement, number of symptoms or foods excluded, age of solid food introduction, type of substitute formula used, duration of breastfeeding, paternal or sibling food allergy history.

Significant differences by SPT were found for duration in weeks of substitute formula use ($p = 0.049$), with the SPT positive group having a longer duration of use. Significant differences were also found between SPT positive and SPT negative groups for maternal allergy (chi square $p < 0.01$), with those in the SPT positive group having higher rates of maternal history of food allergy.

5.5.1.5 Reported symptoms

The median number of symptoms reported by all participants was three (ranging from 0-8). There was no difference found between boys and girls for the number or type of reported symptoms. The number or type of reported symptoms was not correlated to the number of siblings. Participant age and number of symptoms was weakly positively correlated ($rho = 0.24$, $p = 0.016$), with older children reporting more symptoms.

A history of both maternal and paternal food allergy was found to be significantly related to more reported symptoms in the child ($p = 0.023$ and $p = 0.033$ respectively). No difference was found for number of symptoms reported by sibling food allergy history. Reporting of every type of symptom, with the exception of "dry cough" was higher in participants with a maternal history of food allergy (chi square $p < 0.05$ for all). Likewise reporting of all types of symptoms with the exception of wheeze, rash and cough was higher in participants with a paternal history of food allergy (chi square $p < 0.05$). Only colic was more frequently reported in those with a sibling history of food allergy (chi square $p = 0.033$). A higher level of both maternal and paternal education was associated with less reported symptoms in the child (rho = 0.285 $p < 0.01$ and rho = 0.262 and $p < 0.01$ respectively).

The number of allergic symptoms reported was significantly different between the CME and control group ($p < 0.01$). Table 5.4 details the type of symptoms reported by all participants and by dietary exclusion group. The most commonly reported symptom overall was vomiting, reported by 59.4% of all participants. There was a higher rate of reported wheeze ($p = 0.04$), itchy rash ($p < 0.01$), vomit ($p < 0.01$), colic ($p = 0.025$) and "other food related problems" ($p = 0.021$) in the CME group. There was no difference for reported dry cough, diarrhoea, constipation or abdominal distension between dietary exclusion groups.

Table 5.4 Symptoms reported by participants

Symptom	All participants (*N* = 101)	CME group (*n* = 28)	Control group (*n* = 73)
Wheeze or whistling in the chest (%)*	28 (27.7)	14 (50.0)*	14 (19.2)*
Dry cough at night (%)	27 (26.7)	10 (35.7)	17 (23.3)
Itchy rash (%)**	33 (32.7)	16 (57.1)**	17 (23.3)**
Vomiting (%)**	60 (59.4)	25 (89.3)**	35 (47.9)**
Diarrhoea (%)	54 (53.4)	20 (71.4)	34 (46.6)
Constipation (%)	24 (23.7)	8 (28.6)	16 (21.9)
Abdominal distension (%)	11 (10.9)	5 (17.9)	6 (8.2)
Colic (%)*	42 (41.5)	19 (67.9)*	23 (31.5)*
Other food-related problems (%)*	8 (7.9)	5 (17.8)*	3 (4.1)*

*Significant difference between CME and control group chi square $p < 0.05$
** Significant difference between CME and control group chi square $p < 0.01$

The "other food related problems" reported by participants in the CME group were irritable bowel syndrome, urticaria, angioedema, oral pruritus and lethargy. The problems cited in the control group were a rash to melon, tomato and sensitivity to some food odours. None of

the problems mentioned by participants in the control group warranted dietary exclusion or avoidance.

5.5.2 Primary outcome variables: Fussy eating, food neophobia and food preferences

5.5.2.1 Fussy eating

Data was available for all 101 participants. There were no significant differences found for individual subscales of the fussy eating questionnaire by gender or family history of food allergy and no association between fussy eating scores and participant age, maternal/paternal education or occupation status, maternal age or any infant feeding factors.

Differences between the CME and control group for the fussy eating questionnaire are shown in Figure 5.6. There were significantly different scores between the CME and control groups for the “slowness in eating” subscale ($p < 0.01$), with the CME group being slower eaters. No difference was found for the other three subscales (food responsiveness, fussiness and enjoyment of food), although the CME group scored higher for fussiness and lower for enjoyment of food. However when the three negative subscales were combined to give a measure of “avoidant food behaviour”, there were significantly higher scores observed in the CME group ($p < 0.01$). The number of reported symptoms was correlated with higher levels of avoidant eating behaviour, across all participants ($rho = 0.272$, $p < 0.01$). The number of foods excluded was also correlated with slowness in eating ($rho = 0.283$, $p = 0.04$) and avoidant eating behaviour ($rho = 0.345$, $p < 0.01$)

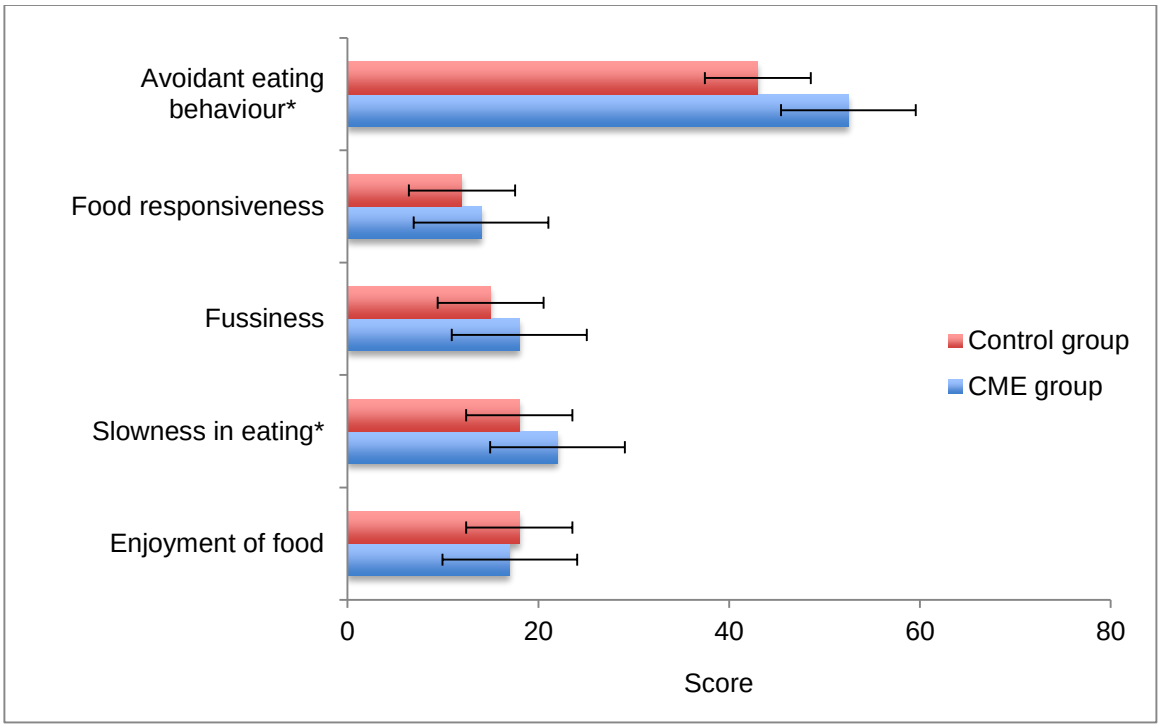

Figure 5.5 Fussy eating questionnaire subscale results by group

*significant difference between CME and control group (Mann Whitney test p < 0.01)

As breastfeeding duration was found to be significantly different between the CME and control groups, a one way between groups analysis of covariance was conducted to compare the avoidant eating behaviour scores between dietary exclusion groups, whilst controlling for breastfeeding duration. Preliminary checks were conducted to ensure that there was no violation of the assumptions of linearity, homogeneity of variances, homogeneity of regression slopes and reliable measurement of the covariate. After adjusting for the breastfeeding duration, a significant difference between the CME and control groups persisted ($p < 0.01$), with dietary exclusion status explaining 11.2% of the variance in avoidant eating behaviour. Breastfeeding duration was not found to be significantly related to avoidant eating behaviour whilst controlling for dietary exclusion group, $F(1, 97) = 12.24$, $p = 0.263$, partial eta squared = 0.013.

Similar to breastfeeding duration, the number of symptoms was found to be significantly different between the CME and control groups. Therefore a one way between groups analysis of covariance was conducted to compare the avoidant eating behaviour scores between dietary exclusion groups, whilst controlling for number of symptoms. Preliminary checks were conducted to ensure that there was no violation of the assumptions of linearity, homogeneity of variances, homogeneity of regression slopes and reliable measurement of the covariate. After adjusting for the number of symptoms, a significant difference between the CME and control groups persisted ($p < 0.01$), with dietary exclusion status explaining 7.8% of the

variance in avoidant eating behaviour. The number of symptoms was not found to be significantly related to avoidant eating behaviour whilst controlling for dietary exclusion group, $F(1,98) = 1.45$, $p = 0.230$, partial eta squared = 0.015.

The influence of positive family history of food allergy on avoidant eating behaviour was investigated as it was found to be significantly different between the CME and control groups in preliminary analyses. A two way between groups analysis of variance was conducted to compare the avoidant eating behaviour scores between dietary exclusion groups, whilst controlling for family food allergy history. Preliminary checks were conducted to ensure that there was no violation of the assumptions of linearity, homogeneity of variances, homogeneity of regression slopes and reliable measurement of the covariate. After adjusting for family history of food allergy, a significant difference between the CME and control groups persisted ($p = 0.01$), with dietary exclusion status explaining 10.4% of the variance in avoidant eating behaviour. Maternal, paternal or sibling food allergy was not significantly related to avoidant eating behaviour whilst controlling for dietary exclusion group $F(1,95) = 0.439$, $p = 0.509$, partial eta squared = 0.005 for maternal; $F(1,95) = 0.485$, $p = 0.488$, partial eta squared = 0.005 for paternal; and $F(1,95) = 0.246$, $p = 0.236$, partial eta = 0.03 for sibling.

Finally, a two-way between-group analysis of variance was conducted to explore the impact of predominant type of infant food consumed (readymade or homemade) and dietary exclusion group on avoidant eating behaviour. The interaction effect between dietary exclusion group and food type was not statistically significant $F(2,93) = 0.74$, $p = 0.47$. There was a statistically significant main effect for dietary exclusion group $F(2,93) = 5.57$, $p = 0.02$; however the effect size was small (partial eta squared = 0.57). The main effect for baby food type did not reach statistical significance $F(2, 93) = 0.49$, $p = 0.95$.

5.5.2.2 Food preference

Food preference data was available for all participants. Total liking for all foods was not found to be associated with age, maternal age, number of symptoms, growth or any infant feeding variables. Liking for all foods was inversely related to food fussiness (rho = -0.473, $p < 0.01$) slowness in eating (rho = -0.340, $p < 0.01$) and food neophobia (rho = 0.583, $p < 0.01$), but positively correlated to enjoyment of food (rho = 0.314, $p < 0.01$). Total liking for all foods ($p < 0.01$) and liking for a number of food categories were rated more highly by girls than boys; specifically sweet foods ($p = 0.035$), dairy foods ($p = 0.049$), eggs ($p < 0.01$) and vegetables ($p < 0.01$). Liking for food categories was not associated with age, with the exception of fish, which was inversely associated with age (rho = -0.255, $p = 0.01$).

Median liking scores for each food category is shown in Table 5.5. Because each category had a different number of foods, the median scores have been subdivided by the number of foods in each category. The most preferred category overall was the sweet and fatty foods category and the least preferred category was vegetarian substitute, followed by vegetables. No difference was found between the CME and the control group for any category of food.

Table 5.5 Preference score for each food category

Category	All (*N* = 101)	CME group (*n* = 28)	Control group (*n* = 73)
All foods combined (119)	4.15	4.23	4.10
Meat (9)	4.55	4.50	4.50
Fish (4)	3.75	3.65	3.75
Fruit (14)	4.78	4.67	4.78
Vegetables (25)	3.52	3.64	3.52
Starchy carbohydrates (13)	4.07	4.07	4.15
Eggs (4)	4.25	4.37	4.25
Processed meat (9)	3.66	3.72	3.66
Vegetarian Substitutes (5)	1.12	1.12	1.0
Dairy (16)	4.18	4.06	4.21
Sweet & fatty foods (20)	5.60	5.60	5.57

Median score displayed. Number of foods in each category is in brackets. Higher score = higher preference and vice versa.

However, looking at individual foods within the dairy category (and other milk containing foods such as chocolate), significant differences were found between the CME and control groups for a number of individual foods, with the control group rating them more positively. These foods are show in Table 5.6.

Table 5.6 Preference rating for individual dairy foods

Dairy foods rated significantly worse by CME group than control group		Dairy foods with no significant difference in rating between groups	
Food	p value	Food	p value
Full fat milk	0.004*	Semi skimmed milk	0.164
Butter	0.043*	Skimmed milk	0.247
Cream	0.016*	Margarine	0.243
Ice cream	0.030*	Custard	0.153
Chocolate	0.024*	Yoghurt	0.168
		Hard cheese	0.853
		Processed cheese	0.162
		Cream cheese	0.553
		Soft cheese	0.808
		Low fat cheese	0.459

Mann Whitney test p values shown. *p < 0.005

5.5.2.3 Food neophobia

Data was available for all 101 participants. The median food neophobia score was 34 (ranging from 10-70). The minimum and maximum possible scores on this questionnaire are 10 and 70 respectively. When the neophobia data was split into two categories of low and high (above and below the median score respectively) there was no significant difference for age, gender, family history of food allergy, parental occupation/education or infant feeding status. Those in the higher food neophobia category had significantly higher scores for slowness in eating, fussiness and avoidant eating behaviour and lower scores for enjoyment of food ($p < 0.01$ for all). The correlation between food neophobia and the fussiness subscale of the CEBQ was very strong (rho = 0.836, $p < 0.01$), with the other subscales of enjoyment and slowness also showing moderate correlations (rho = 0.448, rho = 0.414 $p < 0.01$ respectively).

There was no difference for food neophobia score by gender or family history of food allergy and no association between food neophobia score and participant age, parental education/occupation status, maternal age or any infant feeding factors. There was no difference between CME and control group, with the CME group scoring a median of 36 (12-60) and the control group scoring a median of 34 (10-70). There was no association found for number of symptoms or number of foods excluded.

Looking at all participants, food neophobia was inversely correlated with total liking for

all foods (rho = 0.583, $p < 0.01$), indicating children with lower levels of neophobia like more foods overall. It was also inversely correlated with all sub categories of foods, as shown in Table 5.7, with all associations being significant at a level of $p < 0.01$. The strongest association was shown for vegetables (rho = -0.504, $p < 0.01$). Food neophobia was not correlated with any macro or micronutrient intake or growth measurement.

Table 5.7 Correlation coefficients for food neophobia scores and liking of food categories

Food category	Spearmann rho	p value
Sweet and fatty foods	-0.350	0.000
Dairy	-0.274	0.006
Vegetarian foods	-0.280	0.004
Processed meat	-0.495	0.000
Eggs	-.0.491	0.000
Starchy foods	-0.455	0.000
Vegetables	-0.504	0.000
Fruit	-0.264	0.008
Fish	-0.316	0.001
Meat	-0.355	0.000

5.5.2.4 Fussy eating during early childhood and association to outcome variables

Respondents were asked if their child had been a fussy eater at 6-12 months old, 1-2 years old and 2-3 years old. The results are shown in Figure 5.7. Fussy eating status increased with each age bracket, but there was no differences observed according to gender, family history of food allergy, breastfeeding history or solid food introduction. Participants reported to be fussy eaters at 6-12 months, 1-2 years and 2-3 years were reported to have significantly more symptoms ($p < 0.01$, $p = 0.018$ and $p = 0.043$ respectively). Overall a greater percentage of children in the CME group were reported to be fussy eaters at each age bracket than the control group. The difference between groups was significant at 6-12 months and 1-2 years ($p < 0.01$ and $p = 0.022$ respectively).

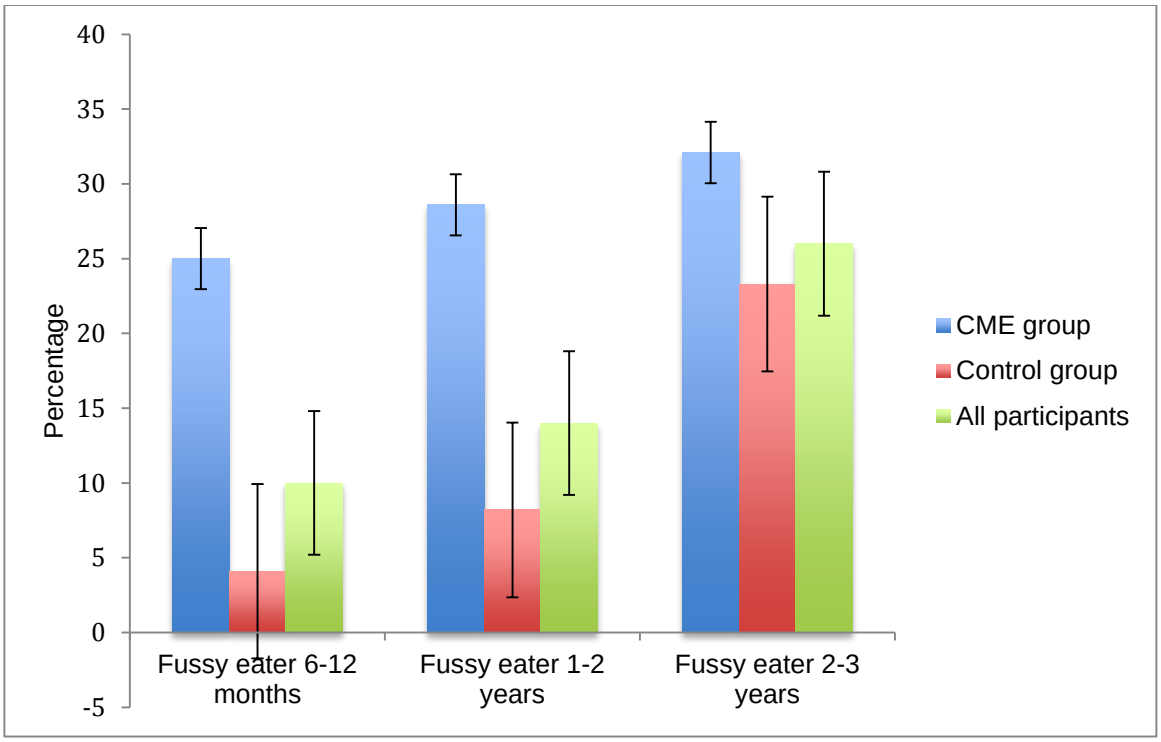

Figure 5.6 Percentage of parents reporting child was a fussy eating in early life

Participants who were reported to be fussy eaters in early childhood were found to have significantly higher levels of avoidant eating behaviour at present ($p = 0.027$, $p < 0.01$ and $p < 0.01$ respectively). Additionally, those who were reported to be fussy eaters at 1-2 years old and 2-3 years old were found to have significantly lower scores for food enjoyment ($p = 0.032$ and $p < 0.01$) and higher levels of food neophobia at present ($p < 0.01$ and $p < 0.01$). Results are shown in Table 5.8.

Table 5.8 Differences in current levels of food neophobia and fussy eating per fussy eating category in early life

	Fussy eater at 6 months			**Fussy eater at 1-2 years**			**Fussy eater at 2-3 years**		
	Yes	No	p	Yes	No	p	Yes	No	p
Food neophobia score	36.5	34	0.421	43	33.5	0.041*	46.5	32	0.000*
Fussiness subscale	18	15	0.084	19	15	0.013*	19	14.5	0.001*
Enjoyment of food	15.5	18	0.121	15	18	0.032*	15	18.5	0.003*
Slowness of eating	19.5	19	0.523	21	18	0.036*	21	18	0.007*
Food responsiveness	16	12	0.424	11	12	0.735	12	12	0.568
Avoidant eating	53	45	0.027*	54.5	44.5	0.005*	53	44	0.003*

*significant difference in Mann Whitney test $p < 0.05$

There was no difference for food preference scores for any food category whether a child was reported to be a fussy eater at 6-12 months or 1-2 years. However those who were reported to be fussy eaters at 2-3 years old had lower food preference scores for all food categories and the differences were significant for eggs ($p < 0.01$), vegetables ($p < 0.01$), fruit ($p = 0.012$), starchy carbohydrates ($p = 0.043$), processed meat ($p < 0.01$) and meat ($p = 0.014$).

5.5.3 Secondary outcome variables: taste preference, nutritional intake and growth

5.5.3.1 Taste preference

Five participants did not complete the taste preference test, therefore data was available for 96 participants. Results of taste preference test are shown in Table 5.9.

Table 5.9 Taste preference test results

	All participants (*N* = 96)	CME group (*n* = 24)	Control group (*n* = 72)	p value
Sweet	6	5	6	0.077
Sour	3	3	3	0.766
Bitter	5	6	5	0.050
Water	7	7	6	0.620
Umami	3	2	3	0.359
Salty	3	3	3	0.632

Median scores shown. Higher scores indicate a better perceived taste and vice versa.

The most preferred taste overall was water (median score 7 = “good”), followed by sweet (median score 6 = “just a little good”). The least preferred tastes overall were umami, salty, and sour, each scoring a median score of 3 (corresponding to a hedonic rating of “bad”). Boys rated sweet ($p = 0.037$), umami ($p = 0.012$) and salty ($p = 0.015$) tastes significantly worse than girls. Sweet taste preference was positively correlated with age of participant ($rho = 0.292$, $p < 0.01$).

There was no association found for any taste preference and any growth measurement or infant feeding variable, number of symptoms or number of foods excluded. There was no significant difference between the CME group and control group for any taste although, there was a trend almost reaching significance with those in the CME group rating the bitter taste higher than the control group ($p = 0.050$). Within the CME group, bitter taste preference was not found to be significantly correlated with age of introduction of substitute formula, duration of substitute formula usage, age of introduction of solids, duration of breastfeeding or number of foods excluded. Bitter taste preference did not differ per type of substitute formula used,

however the study was not sufficiently powered to detect a difference between formula groups. Within the CME group bitter taste preference was positively correlated with “enjoyment of food” (rho = 0.450, p = 0.027), but not correlated with any other measure of fussy eating, food neophobia, or food preference category. This correlation was not evident when looking at all participants as a whole.

5.5.3.2 Nutritional Intake

Food diaries were returned for 64 participants (63.3%); 17 from the CME group (60.7%) and 47 (74.6%). from the control group. There was no difference between those who did and did not return the food diary for age, gender, parental education or occupation status, maternal age, food exclusion history, family history of food allergy, number of symptoms/foods excluded, growth measurements, fussy eating, food neophobia or food liking scores.

A summary of food diary nutritional analysis is shown in Table 5.10. Median values and % of the Reference Nutrient Intake (RNI) are shown in brackets. Using the average requirements for the 7-10 year old age bracket as a guide, overall participants met the Estimated Average Requirement (EAR) for all nutrients. Intakes of vitamin B6, vitamin B12 and vitamin C seemed particularly high for all participants (248% of RNI, 273% of RNI and 244% of RNI respectively). Intakes of some minerals appeared suboptimal (iron 72% of RNI, zinc and magnesium both 74% of RNI), however as already stated, they were above the EAR. There is no recommended intake for vitamin D for children older than five years old. However the mean intake of 1.83 mcg seems extremely low, given that the RNI for children under five years old is 7.5 mcg. Boys had significantly higher intakes than girls for protein, sodium, iron, zinc, magnesium, iodine and phosphate ($p < 0.05$ for all).

Looking at dietary exclusion groups separately, the CME group’s intake of zinc and iodine, was below the EAR, but above the Lower Reference Nutrient Intakes (LRNI) of 4mg and 50.8 ug respectively. The control group met the EAR for all nutrients. Both groups had remarkably similar intakes of energy, protein, fat, saturated fat and vitamin D. The control group had higher intakes of calcium, iron, zinc, magnesium, iodine, phosphorous, thiamine, riboflavin and vitamin B12. The difference was only significant for iodine ($p< 0.01$) and riboflavin ($p = 0.029$). The CME group had higher intakes of fibre, sodium, selenium, vitamin A, vitamin C and vitamin E. The difference was significant for sodium ($p = 0.018$) and selenium ($p = 0.016$).

Table 5.10 Median intakes of selected nutrients from food diary analysis

	All (*N* = 64)	CME group (*n* = 17)	Control group (*n* = 47)
Energy (kcal)	1687 (82%)	1668 (85.0%)	1688 (82%)
Protein (g)	62.1 (156%)	62.4 (152%)	62.05 (156%)
Fat (g)	63.8 (84%)	63.9 (83.0%)	63.8 (87.0%)
Saturated fat (g)	24.85 (107%)	24.9 (107%)	24.8 (104.5%)
Fibre (g)	14.3 (N/A)	15.4 (N/A)	13.9 (N/A)
Sodium (mg)*	2252 (155%)	2819 (176%)*	2166 (144.0%)*
Calcium (mg)	704.5 (84%)	587 (74.0%)	717 (88.5%)
Iron (mg)	9.1 (72%)	8.2 (61.0%)	9.31 (75.5%)
Zinc (mg)	6.39 (74%)	5.3 (66.0%)	6.5 (75.0%)
Selenium (mcg)*	34.85 (80%)	42.4 (98.0%)*	34.2 (78.0%)*
Magnesium(mg)	194 (74%)	188.0 (74.0%)	194.0 (75.0%)
Iodine (mcg)*	108 (86.5%)	67.1 (55.0%)*	118.4 (93.0%)*
Phosphorous (mg)	1077 (164%)	986.5 (158.5%)	1082 (165%)
Vitamin A (mcg)	517 (103%)	538 (107%)	479 (95.8%)
Thiamin (mg)	1.37 (175%)	1.29 (175%)	1.40 (175%)
Riboflavin (mg)*	1.28 (116%)	1.09 (93%)*	1.42 (124%)*
Niacin(mg)	15.2 (114%)	15.9 (136%)	15.19 (107.5%)
Vitamin B6 (mg)	1.54 (248%)	1.58 (248%)	1.52 (252%)
Vitamin B12 (mcg)	3.0 (273%)	2.1 (187%)	3.04 (291.5%)
Folate (mcg)	192 (104%)	185 (101%)	195 (104%)
Vitamin C (mg)	84.0 (244.0%)	114 (325.0%)	78.0 (236%)
Vitamin D (mcg)	1.83 (NO DRV)	1.92 (NO DRV)	1.83 (NO DRV)
Vitamin E (mg)	6.32 (NO DRV)	7.97 (NO DRV)	6.31 (NO DRV)

%Reference nutrient intake is shown in brackets. *significant difference between groups using a Mann Whitney test $p < 0.05$

As the intake of some nutrients was found to be significantly different between boys and girls, a two way between groups ANOVA was conducted to compare sodium and iodine intakes between dietary exclusion groups, whilst controlling for gender. Preliminary checks were conducted to ensure that there was no violation of the assumptions of linearity, homogeneity of variances, homogeneity of regression slopes and reliable measurement of the covariate. After adjusting for the gender, a significant difference between the CME and control groups

persisted for iodine intake ($p < 0.01$), with dietary exclusion status explaining 12.5% of the variance in iodine intake. Gender was not found to be significantly related iodine intake whilst controlling for dietary exclusion group ($p = 0.068$, partial eta squared = 0.057). In terms of sodium intake, the same trend emerged. After adjusting for the gender, a significant difference between the CME and control groups persisted ($p < 0.01$), with dietary exclusion status explaining 10.4 % of the variance in sodium intake. Gender was not found to be significantly related sodium intake whilst controlling for dietary exclusion group ($p = 0.119$, partial eta squared = 0.029).

5.5.3.2.1 Dietary supplements

Dietary supplement composition was included in the food diary nutritional analysis. In total 21 (20.7%) participants took dietary supplements, 7 (25%) from the CME group and 14 (19.2%) from the control group. Two of the CME group took calcium/vitamin D supplements, with the remainder taking multivitamin/mineral combinations. All 14 of the control group took multivitamin/mineral supplements. Children who took dietary supplements had a significantly higher intake of fibre ($p = 0.017$) and a significantly %RNI of magnesium ($p < 0.01$), iron ($p < 0.01$), vitamin B6 ($p < 001$), folate ($p = 0.01$) and vitamin C ($p = 0.037$).

5.5.3.2.2 Healthy eating

Respondents were asked “how much attention was paid to their child’s diet in terms of healthy eating”. Proportionately more respondents in the CME group than the control group responded “a great deal” (64.3% and 47.9% respectively), however the difference was not statistically different between groups. Energy intake (kcal) was found to be significantly lower in those with a higher awareness of healthy eating (78% compared to 90.5% of requirements, $p = 0.015$), but there was no difference for any other nutrient.

Those who reported to pay “a great deal” of attention to healthy eating had higher liking scores for fruit, vegetables, starchy carbohydrates, eggs, dairy and fish, but lower liking scores for meat and processed meats. The differences were not significantly different between groups. There was no difference by healthy eating status for fussy eating or food neophobia.

5.5.3.3 Growth measurements

Details of birth weight, current weight, height, BMI and waist circumference are shown in Table 5.11. Current measurements were available for all but one participant. Weight for age percentile was calculated manually using a UK growth chart, as it was not possible to calculate this using the WHO Anthro plus software for children aged above 10 years old. 100% weight for age

corresponds to the 50th centile, therefore the median weight for age percentile of 106.7 is relatively normal.

Height percentile was calculated using the WHO Anthro plus software and for this value, 50% corresponds to the 50th centile. Therefore the median percentile of 65.2% indicates that participants were slightly above average height, compared to other children in the UK of the same age. Likewise the median BMI centile of 58.15% can be considered relatively normal. Overall participants had very high waist circumference centiles (median of 98.8%). In total, 19 participants could be classified as overweight or obese (BMI > 91st centile), with no difference observed for age, gender, number of siblings or parental education/occupation. There was also no difference between healthy weight and overweight/obese children for liking of any food category, fussy eating, food neophobia, nutritional intake or taste preference. There was a significant difference identified for food responsiveness ($p < 0.01$), indicating that those in the overweight/obese category have a tendency to respond to appetite in a maladaptive manner.

Comparing dietary exclusion groups, eight participants in the CME group and 11 participants in the control group were classified as overweight/obese. Although there is proportionately nearly twice as many of the CME group in the overweight/obese category (28.6%) compared to the control group (15%), the difference was not statistically significant. There was no difference between dietary exclusion groups for any of the measurements.

Table 5.11 Anthropometric measurements of participants

	All (*N* = 101)	CME group (*n* = 28)	Control group (*n* = 73)
Birth weight (kg)	3.39 (1.71-4.59)	3.4 (1.71-4.08)	3.37 (2.26 – 4.59)
Weight (kg)	38.8 (20.1 – 74.5)	38.9 (22.2 – 74.5)	38.7 (20.1 – 69.9)
Height (cm)	147.7 (118.8 – 165.5)	143.3 (120.6 – 163.1)	148.0 (118.8 – 165.5)
Weight for age (%)	106.7 (72.5 – 201.3)	103.8 (77.8 – 201.3)	107.4 (72.5 – 174.75)
Height centile (%)	65.2 (3.2 – 97.8)	64.5 (3.4 – 97.5)	66.9 (3.2 – 97.8)
BMI (kg/m$^{2)}$	17.59 (11.9 – 30.1)	17.59 (15.1 – 30.1)	17.6 (11.9 – 29.26)
BMI centile (%)	58.15 (2.0 -99.9)	56.1 (15.9 – 99.8)	59.8 (2.0 – 99.9)
BMI z score (SD units)	0.20 (-2.79 – 2.99)	0.15 (-1.0 – 2.92)	0.25 (-2.79 – 2.99)
Waist (cm)	58.95 (46.2 – 90.3)	58.95 (48.3 – 79.0)	58.95 (46.2 – 90.3)
Waist centile (%)	98.8 (84.2 – 145.0)	97.85 (87.2 – 135.0)	99.1 (84.2 – 145.0)

Median values shown. Minimum and maximum values in brackets.

5.5.4 Differences between participants according to recruitment method

In order to assess whether there were any differences between participants dependent on whether they were recruited from the FAIR study, PIFA study or NHS clinics, Kruskal-Wallis tests were undertaken, followed by post-hoc Mann Whitney U test with Bonferroni adjustment. No differences were found for any of the main outcome variables across the three recruitment methods for fussy eating, food neophobia, food preferences, growth or taste preferences. There was a significant difference in both maternal and paternal education level across the three different groups (χ^2 (2, n = 100) = 19.63, p < 0.01) and (χ^2 (2, n = 99) = 9.142, p < 0.01) respectively. Mothers and fathers in the PIFA study had higher educational levels than the FAIR study (p < 0.01 and p < 0.01 respectively) and NHS participants (p = 0.019 and p = 0.044). As to be expected, there was also a significant difference in the age of the children (χ^2 (2, n = 101) = 46.28, p < 0.01), PIFA participants were significantly younger than both NHS and FAIR participants (p < 0.01 and p < 0.01 respectively). No difference was found for any infant feeding variable, with exception of age of introduction of solid food (χ^2 (2, n = 95) = 20.65, p < 0.01), which occurred earlier in the FAIR participants than the PIFA participants (p < 0.01).

Looking at the CME group only, differences were observed between the three recruitment centres for number of foods excluded (χ^2 (2, n = 28) = 7.609, p = 0.022), age of specialised formula introduction (χ^2 (2, n = 28) = 7.296, p = 0.026) and number of dietetic contacts (χ^2 (2, n = 28) = 9.421, p < 0.01). No difference was identified between recruitment centre for number of symptoms reported or duration of substitute formula use. Participants from the FAIR cohort were initiated on a substitute formula earlier than those in NHS clinics (p < 0.01), but no difference was seen between FAIR and PIFA cohorts. Children recruited from NHS clinics excluded significantly more food allergens than those from the FAIR cohort (p = 0.012). In terms of dietetic contacts, participants from the PIFA cohort had significantly more contacts than the FAIR study (p = 0.010) and NHS participants (p = 0.013).

5.5.5 Food challenge data

As part of the protocols for both the FAIR and PIFA studies, children with reported adverse food reactions to cows' milk underwent a diagnostic food challenge following a period of dietary elimination. Children recruited from NHS clinics did not have food challenges as part of their routine clinical care, so were excluded from this part of the analysis.

In total 6/23 (26%) of the CME group had a positive challenge to cows' milk and 17/23 (74%) had a negative challenge. Mann Whitney and chi square tests were undertaken to determine if there was any difference in outcome variables between those who had positive

and negative food challenges. There was no significant difference in any primary or secondary outcome variable between challenge groups (fussy eating, neophobia, food preferences, taste preference, nutritional intake or growth). There was no significant difference between positive and negative challenge groups for type or number of allergic symptoms reported or any infant feeding variable. Those who had positive challenges excluded significantly more foods ($p = 0.015$), had a longer duration of CME ($p = 0.02$) had a greater number of dietitian contacts ($p < 0.01$) and were more likely to be fed an EH casein formula than a soya formula ($p = 0.025$). There was also a higher rate of maternal food allergy history in those with positive challenges ($p < 0.01$), but no difference in the rate of paternal or sibling food allergy history.

5.6 Discussion

This study set out to compare the eating habits of two groups of school-aged children: one group who had consumed a CME diet for suspected CMA as infants and one group who had consumed an unrestricted diet as infants, but who are all now consuming unrestricted diets. As the majority of participants (96/101) were recruited from two large birth cohort studies it was possible to measure the effect of infant feeding variables on current eating behaviours using prospectively collected data. There were two primary objectives set for this study:

1. To determine food preferences in children who consumed a CME diet during infancy compared to a control group who consumed an unrestricted diet.
2. To determine the degree of fussy eating and food neophobia in children who consumed a CME diet in infancy compared to a control group who consumed an unrestricted diet.

Overall the CME group rated several dairy foods (butter, cream, chocolate, full fat milk and ice cream) significantly lower than the control group, although there were no significant differences seen for the overall dairy category or for any other category of food. Significant differences in food preference were also found for gender, with girls rating several food categories higher than boys.

In terms of fussy eating, the CME group scored significantly higher on “slowness of eating” and on the combined “avoidant eating behaviour” construct. There was no difference according to gender or infant feeding, but a higher number of foods excluded and symptoms were associated with more negative eating behaviour. There was no difference between groups for food neophobia, or any association observed with infant feeding. Being reported to be a fussy eater in early life was strongly predictive of current eating behaviour and current food preference.

The secondary objectives of the study were to investigate and compare taste preferences, nutritional intake and growth between dietary exclusion groups. No significant difference was found between groups for taste preference, with the exception that the CME group had a higher preference for bitter taste *approaching* significance ($p = 0.05$). In terms of nutritional intake, significant differences were found between dietary exclusion groups for some minerals (selenium, iodine, riboflavin and sodium), but macronutrient intakes were remarkably similar between groups. No significant differences were found between groups for any growth measurement, although nearly twice as many participants in the CME group were classified as overweight/obese compared to the control group. It was noted that a high median waist circumference was evident in both groups of participants.

5.6.1 Demographic characteristics of sample

This study recruited participants from two different, yet geographically close, locations in the South of England (Isle of Wight and Winchester). The predominant ethnic group of white British recruited for this study is broadly reflective of the population in Hampshire, which is 89% white British and 4% white other compared to 80.5% white British and 4.4% white other nationally (Office for National Statistics, 2012). The parents of participants were well educated with over a third having a graduate or postgraduate qualification. The age of the participants is relatively broad, spanning almost seven years, however all children in this age bracket are school age, able to read and write therefore able to complete simple questionnaires and take part in measurements. The relatively high level of maternal food allergy history (23% overall) could be due to the fact that those with a food allergy history were more likely to take part in this follow up study.

5.6.2 Infant feeding characteristics

The infant feeding data was collected prospectively for all participants from the original birth cohort studies. For a small minority of participants ($n = 5$) who were recruited from the NHS allergy clinic records, the answers were based on recall. Only the questions regarding “age of introduction of lumpy and finger foods” and the “predominant type of weaning food” were not collected in the birth cohort studies, so were based on recall for all participants. This therefore removes the problem of recall bias to a large extent.

The original FAIR and PIFA studies took place in 2001-2002 and 2006-2008 respectively, therefore the infant feeding practices at the time are of relevance and the results may need to be interpreted in context of what was the norm at the time. The fact that the majority of participants had been breastfed at one stage (78.0%) compares favourably to

national data at the time 66% in 2000 (Hamlyn, Brooker, Oleinikova, & Wands, 2002) and 78% in 2005 (Boiling, Grant, Hamlyn, & Thornton, 2005). However significantly more of the control group had ever been breastfed compared to the CME group (chi square p = 0.02) and they were breastfed for longer (chi square p = 0.017). All of the CME group had been given formula milk, compared to 87.5% of the control group. No significant difference was found between groups for ever been fed formula milk or age at introduction of formula milk.

The median age of solid food introduction was 16 weeks in both CME and control groups, however the FAIR participants introduced solids significantly earlier than participants recruited from the PIFA cohort or NHS clinics. At the time of the FAIR study in 2001/2002, the advice was based on the COMA report of 1994 recommending "the majority of infants should not be given solid foods before the age of four months and a mixed diet should be offered by the age of six months". At the time of the PIFA study (2006/2008), the UK Department of Health had revised the advice based on WHO recommendations of 2001 (World Health Organisation, 2002), therefore the advice was to introduce solids around six months.

Looking at previous publications which describe the infant feeding pattern of the whole FAIR cohort study (n = 969) (Venter et al., 2009), more than a quarter (27.3%) of mothers had introduced solids into the infants diet by three months of age, 82.1% before 17 weeks and all mothers by six months. Therefore, it can be said that both groups introduced solid foods earlier than was recommended at the time. The predominant type of baby food used differed between groups (chi square p = 0.018), with a greater proportion of those in the CME group using readymade baby food (14.3% compared to 1.4% of the control group. A recent qualitative study, also from the South of England, indicated that parents of infants with food allergy may perceive commercial readymade baby food as “safer”, which may explain why they were used more frequently (Venter, Maslin, & Dean, 2015). This trend of increased use of readymade baby food in infants with CMA was also previously reported and discussed in chapter four of this thesis.

Two participants had a very high age of introduction of lumpy and finger foods (approximately four years old), which were outliers in the dataset. One participant was in the CME group and one in the control group. It was decided to retain these values in the analysis. Neither child now has any difficulty with textured foods, therefore it was important to include their data as it reflects the variation in the sample and the natural differences that occur in children’s feeding development

5.6.3 Dietary exclusion

Infants in the CME group had cows' milk excluded from their diet and initiated a substitute formula for CMA at a relatively early age (11.5 weeks). However it must be underlined that the majority of these infants (23/28) were enrolled in a large epidemiological study of childhood food allergy, therefore they were advised to report any adverse reaction to foods, were monitored with questionnaires closely and had regular access to an allergy dietitian and research nurses. The most commonly used formula in the CME group was soya (50%), which was frequently used at the time of the FAIR study (2001/2002). New recommendations advising against the use of soya formula in the management of CMA were published in 2003 (Committee on Toxicity of Chemicals in Food, 2003). Hence the most commonly used formula within the PIFA cohort, who were born in 2006/2008, was an EH casein formula.

The relatively low usage of Amino Acid Formula (AAF) (10.7%) is of note. Although evidence based guidance suggests that only 10% of infants presenting with CMA will require an AAF (Høst & Halken, 2004), as was seen in chapter four of this thesis AAF are sometimes inappropriately prescribed and used more frequently than necessary. However as the infants in the FAIR and PIFA studies were seen by specialist allergy teams who had extensive experience in the management of CMA, it is not surprising that the hydrolysed formulas were appropriately prescribed, with only a minority of participants (25%) requiring a change of formula.

The use of a substitute formula for a longer period of time in the SPT positive subgroup is not surprising, as it is conceivable that they had more severe presentations and were reluctant to have a food challenge at an earlier stage. The fact that those recruited from NHS clinics excluded more foods than those in the PIFA and FAIR studies may be because participants in the birth cohort studies received more frequent dietetic input with regular SPTs as part of the study protocols. The exclusion of more foods, a longer duration of substitute formula and greater number of dietetic contacts in those with a positive challenge compared to a negative food challenge is also to be expected as those who had a positive challenge may have been more anxious to reintroduce foods.

5.6.4 Symptoms

Typical allergic symptoms were reported in both control and CME groups, although as expected the number was significantly higher in the CME group. The presence of some allergic symptoms in the control group, may be due to misunderstanding and misinterpreting terms (e.g. precise definitions of "wheeze" and "diarrhoea") and also due to the fact that many of the

symptoms typically occur in normal developing children as a result of common childhood illnesses. For example, asking about a history of “vomiting” could be perceived as gastro-oesophageal reflux due to CMA or normal posseting of feeds, or vomiting due to an illness, hence why it was reported by over half (59.4%) of all participants. Also, as previously mentioned, both CME and control participants were regularly monitored and questioned regarding allergy symptoms as part of the birth cohort studies so were likely to be more aware of them due to frequent prompts. The higher rate of symptoms in those with a maternal and paternal history of food allergy symptoms was also reported in the previous chapter and is likely due to heredity or a heightened awareness of allergic symptoms.

The similarity in symptoms reported between those with positive and negative food challenges to milk may be due to the time lag effect between presentation with symptoms and undertaking the challenge or the challenge criteria. In the PIFA study, any symptoms occurring later than two hours after the last challenge dose (e.g. colic) were not considered to be a positive challenge. The mean time lag was four months, therefore it may be that by the time the food challenge took place, the allergy had already started to be outgrown (Schoemaker et al., 2015) . On the contrary, it could be that the children in the CME group who had negative challenges did not have CMA and their symptoms were misperceived and over-reported by parents.

5.6.5 Fussy eating and slowness in eating

The significant difference observed between dietary exclusion groups for avoidant eating behaviour using prospectively collected data is a novel finding. It demonstrates a longitudinal effect of adapting the infant diet, persisting approximately 7-10 years, even whilst controlling for breastfeeding duration and number of allergic symptoms. The moderate correlations observed between both number of symptoms and number of foods excluded and worse eating behaviour is also a novel finding. Although the fussy eating measure was based on parent report rather than child report or direct observation of eating, as previously discussed the questionnaire used is validated and robust. Two studies investigating fussy eating in school-aged children, have recorded food allergy history as a variable, but both collected the data retrospectively, there was no confirmation of food allergy diagnosis and no details of foods excluded or symptoms are reported. Xue et al.’s study of school children in China (2015) included a small subgroup of children who had a history of food allergy, reporting that 9.2% of those with a food allergic history were picky eaters compared to 6.5% of those without a food

allergy history. In contrast, Jacobi et al. (Jacobi et al., 2008) reported that picky and non picky eaters did not differ in terms of number of foods excluded for allergy.

The finding that those in the CME group are slower eaters is of note. Mealtime duration is associated with problem eating behaviour with prolonged mealtimes known to worry parents (Crist & Napier-Phillips, 2001; Powers et al., 2002). Slower eating times have been reported in toddlers who are fussy eaters (Reau, Senturia, Lebailly, & Christoffel, 1996; Wright et al., 2007). It is thought that delaying eating by talking may be used as a distraction to avoid finishing meals. However it must be highlighted that mealtime duration was not measured directly, but assessed using an indirect questionnaire measure. Adamson et al. (2015) investigated mealtime duration in children aged 2-5 years with and without feeding difficulties using both parent report and direct mealtime observation. They found parents to be accurate reporters of mealtime duration. Although meal time duration was found to be similar between groups by feeding difficulty status, those in the "problem eater" group engaged in more aversive behaviour and less eating than controls, however Adamson et al. (2015) did not measure nutritional intake during meals. There is a paucity of literature assessing mealtime duration in older children, with most research focused on children under five years old.

It was also interesting that a significantly greater percentage of those in the CME group were reported to be fussy eaters at age six months – two years old. Although this data was based on parent report and is based on recall, it concurs with the data reported in chapter four of this thesis (Maslin et al., 2015). The association between fussy eating in early childhood and current avoidant eating behaviour and food neophobia lends support to the argument that eating behaviour tracks long term from early to later childhood and underlines the importance of establishing acceptable eating behaviours in infancy. Although most research explains fussy eating as a fairly transient behaviour that usually resolves by school age (Cardona Cano et al., 2015), this does not appear to be the case in children who previously consumed a CME diet.

Looking at all participants, it was unusual that we did not find an association between fussy eating levels and any infant feeding variable. Previous research has suggested that breastfeeding can reduce the likelihood of fussy eating, with breastfeeding thought to provide an opportunity for infants to be exposed to a diversity of flavours provided the mother is consuming a varied diet (Forestell & Mennella, 2007; Shim et al., 2011). It has also been suggested that earlier introduction of solids may be associated with fussy eating (Shim et al., 2011), and indeed that infants perceived as fussy are introduced to solid foods earlier (Wasser et al., 2011). We did not find an association in this population. The timing of solid food, lumpy food and finger foods did not differ between the CME and control groups, therefore the

differences we detected in fussy eating cannot be explained by this variable. This may be due to the sample size or differences in methodology and collection of infant feeding data retrospectively by one of the studies (Shim et al., 2011) .

5.6.6 Food preference

The finding that total preference for all foods was inversely correlated to food fussiness and food neophobia is to be expected and validates these measures to a certain extent. The original Cooke & Wardle study (2005) using this questionnaire, also reported that sweet/fatty foods were the most preferred food category and that vegetables were poorly rated. This is a common finding across countries (Nu et al., 1996; Pérez-Rodrigo et al., 2003; Skinner et al., 2002). Cooke & Wardle (2005) also reported gender differences, with girls liking fruit and vegetables more than boys, but boys preferring fatty and sugary foods, meat, processed meat and eggs more than girls. In a study of school children from the USA (n = 1418), girls had significantly higher preference for fruit and vegetables, whilst boys had a significantly higher preference for meat, fish and poultry foods (Caine-Bish & Scheule, 2009). The gender differences in the present study are slightly different; with girls reporting greater preference for sweet/fatty foods, dairy, eggs and vegetables, however the age range of the two studies is different. It is thought that a widening of food repertoire from disliking to liking foods occurs around the age of 10-11 years old (Nu et al., 1996). Cooke & Wardle's study (2005) recruited children from a broad age range (4-16 years), but sub group analysis found that liking for fruit and fatty and sugary foods reached a peak at 8-11 years old. In the present study the only association identified between age and liking was for the fish category.

The categorisation of foods into nine broad categories is to a certain extent arbitrary and is related to food type (e.g. meat, vegetables) not necessarily related to taste or sensory properties. The categories used were similar to that used by Cooke & Wardle (2005) with a few adjustments. The adjustments were discussed and agreed by two research dietitians prior to data collection. For example, Cooke & Wardle (2005) had a number of foods such as tofu and Quorn in an "unclassified" category, which were put in a new "vegetarian foods" category for this study. Milk-containing foods such as pizza and custard were reallocated from the sweet/fatty category to the dairy category. Likewise, Cooke & Wardle (2005) had a separate drinks category, but for the purpose of this study skimmed milk, semi skimmed milk and whole milk were categorised as dairy, with fizzy drinks categorised as sweet/fatty. Despite these changes, the internal stability of most category scales was considered good, with the majority having a Cronbach alpha coefficient > 0.70.

Preference for foods is due to a combination of several properties (e.g. taste, olfaction, texture and temperature). In a study of 4-5 year old children, liking for foods within a category had a certain amount of resemblance (Wardle, Sanderson, et al., 2001), however foods are not preferred due to one simple sensory property (e.g. sweetness, saltiness or creaminess). It is possible therefore that the categorisation of foods into groups may have influenced the results. For example within the fruit category, there are fruits which are typically perceived as sweet (grapes) and those perceived as bitter (raspberries). Likewise, foods were not categorised according to texture, therefore crunchy foods were in the same category as "slimy" foods (e.g. carrot and mushrooms both in the vegetable category). Some food items had several subtypes (e.g. the dairy category consisted of five different types of cheese, but only one type of yoghurt). Also it was arguable that some items in the sweet/fatty category (e.g. chocolate) could have been included in the dairy category and some of the composite foods could have been included in several different categories (e.g. lasagne could have been in meat, starchy carbohydrates or dairy).

Because of all of these limitations with the grouping of individual foods into categories, a sub analysis was carried out on individual milk containing foods, leading to the discovery of significant differences between the CME and control groups. Although ten milk containing foods were found to have no difference in preference ratings between groups (five of which were different types of cheese), significant differences were found for five milk containing foods (full fat milk, butter, cream, ice cream and chocolate). This is particularly surprising, considering that anecdotally chocolate and ice cream are typically well favoured foods by school-aged children. Indeed in Cooke & Wardle's study (2005), chocolate, pizza and ice cream were the three most favoured foods. Chocolate and ice cream were the second and fifth most cited foods in a list of favoured foods amongst French young people also (Nu et al., 1996). Recent national UK dietary data reports that amongst young people aged 4-18 years, mean weekly intakes of chocolate and ice cream are 63-84g and 56-91g respectively (equivalent to approximately two bars of chocolate and 1-1.5 scoops ice cream) (Bates et al., 2014).

The tracking of food preferences from early childhood into later childhood (Skinner et al., 2002) and early adulthood has been reported in the literature (Nicklaus et al., 2004). A high percentage of children's food preferences are formed by the age of 2-3 years old (Skinner et al., 2002) therefore if exposure and liking of these dairy products was not established in infancy due to CMA, it is to a certain extent expected that they are now not liked. It may be that cheese, yoghurt and semi skimmed milk were introduced to the diet of children with resolving CMA as these were deemed to be nutritious foods, yet butter, cream, ice cream, full fat milk and

chocolate were introduced at a later stage as they were perceived to be less healthy or less important, therefore their inclusion into the diet was not prioritised. It is worth emphasising of course, that none of the children recruited into this study reported current adverse reactions to cows' milk, yet it is clear from the results that some have a dislike, which may be a learned association. Milk aversion is well documented in adults with suspected lactose intolerance and it is likely a learned trait caused by experiencing unpleasant side effects such as gastrointestinal illness after consuming a particular food (O'Connor, Eaton, & Savaiano, 2015).

The literature regarding children outgrowing food allergy has not specifically studied the role of food dislike or aversion. Eigenmann et al. (2006) demonstrated that 25% of those who have undergone a negative food challenge do not introduce the food into the diet. This study included several food allergens, with peanut being the allergen least likely to be introduced, however 10% of those with negative challenges to cows' milk did not reintroduce the food. The study did not ask about palatability and liking of the food allergens, however the authors observed that families who had successfully avoided a food for several years did not see the necessity to reintroduce the food, therefore continued avoidance may be due to habit. Flammarion et al. (Flammarion, Santos, Romero, Thumerelle, & Deschildre, 2010) reported that following a negative food challenge, the target food was eaten at least monthly in 83% of cases, but didn't find any difference in the frequency of consumption according to the severity of the initial reaction or the food tested.

In studies that looked specifically at the reintroduction of milk and milk products, Kim et al.'s study (2011) of children who had undergone baked milk challenges, observed that 12% of those who had tolerated baked milk products chose to avoid the food when reviewed five years later. Mostly this was attributed to a fear of recurrence of symptoms and all 12% declined to have a further supervised challenge in hospital. A Swedish study investigating quality of life of children who have outgrown their milk allergy reported that despite development of tolerance, families had continuing nutritional concerns, namely fear of reactions' and 'worrying about the child's health' (Mikkelsen et al., 2015). The authors speculated that the participants may dislike cows' milk which might increase parental nutritional concerns. A study of 210 children in Finland found that although 120 participants had introduced milk products by the age of three years old, with 87% drinking milk, 67% consuming cheese and 45% consuming yoghurt; the total amounts of milk products consumed daily were small and less than the national average (Tuokkola et al., 2010).

Unlike the methodology of Cooke & Wardle (2005), the present study did not take into account the number of foods that were selected as "never tried". Cooke & Wardle (2005)

removed any food that was tried by fewer than 75% of participants from the data analysis and also did separate statistical analyses taking into account foods that were “never tried”. However as theirs was a study of children with a history of normal food consumption, whereas this study specifically was concerned with food preferences of children who were not exposed to a whole food group in infancy, a different methodology is justified. As it has been demonstrated that many food allergic children do not reintroduce food into their diet even once they have had a negative food challenge, it is important to account for foods that have “never been tried” as this is a measure of exposure. Finally, maternal food preferences and food availability was not measured in the present study. It has been reported that foods disliked by mothers tend not to be offered to children (Skinner et al., 2002). It is possible this may have influenced the results and it is acknowledged as a possible limitation. However as this study was specifically focused on the child’s diet, rather than examining family influences of children’s food preferences, it was deemed to be outside the remit of the study.

5.6.7 Food neophobia in school-aged children

The median food neophobia score of 34 is similar to Pliner and Loewen’s (1997) original study that reported mean scores of 31-42 and 37-40 in boys and girls aged 7-12 years old respectively. It is not possible to compare the results directly to other studies of this age group as they have either used a different version of the questionnaire (Cooke, Haworth, & Wardle, 2007; Galloway, Lee, & Birch, 2003) or not published the scores (Falciglia et al., 2000; Mustonen et al., 2012). The strong correlation of food fussiness to food neophobia supports the theory that they are highly interrelated behavioural concepts (Dovey et al., 2008). The correlation of neophobia score with total liking for all foods and each food sub category concurs with previous research (Howard et al., 2012; Russell & Worsley, 2008). However we did not find any association for any aspect of nutritional intake, which is in contrast to other literature. Galloway et al. (2003) and Cooke Wardle & Gibson (2003) both found a lower intake of vegetables was correlated with a higher level of neophobia. However, their studies looked at intake of food groups, rather than nutritional intake, and the age range of participants was younger.

The results of the food neophobia questionnaire demonstrated no difference between dietary exclusion groups. It may be that there is no true difference between groups, or the results could be due to the study not being sufficiently powered or perhaps the age of the participants recruited. Food neophobia is thought to peak at 2-6 years old (Addessi et al., 2005),

yet the participants in this study were between 7-13 years old, therefore it may be that food neophobia existed at an earlier age, but has been outgrown.

As mentioned in previous chapters, the existing research on food neophobia and previous dietary exclusion for food allergy is sparse, with only one study identified in an extensive literature search. Rigal et al. (2005) compared food neophobia levels in children who have outgrown their food allergy to that of a sibling (mean 7-9 years old). They concluded that previously food allergic children are more reluctant to try new foods than their non-allergic sibling and that food neophobia is worsened in the case of late diagnosis and when the preparation of meals was perceived as difficult. The reason that Rigal et al. (2005) found a significant difference between groups, whereas the present study did not can easily be explained by several study design differences. For example, Rigal et al. recruited participants with a history of both single and multiple food allergies, the studies took place in a different country with potentially different food cultures and different questionnaires were used. Finally Rigal et al. used a sibling as a comparison, rather than an unrelated participant.

In terms of family members and the contribution of the shared environment to food neophobia levels, a study of 8-11 year old twins reported that food neophobia is a highly heritable trait (Cooke, Haworth, & Wardle, 2007). Other studies have also suggested there is a correlation between maternal and child food neophobia (Galloway et al., 2003; Skinner et al., 2002), therefore it is possible food neophobia is influenced by family characteristics more so than food allergy status. As we did not measure maternal food neophobia or any maternal food measures, it is difficult to speculate whether this is the case.

5.6.8 Nutritional Intake

The number of food diaries returned was similar to that of other studies Christie et al. (2002) reported that 66% and 64% of food allergic and control participants respectively returned food diaries. Similarly Flammarion et al. (2011) reported a return rate of 65% and 55% for food allergic and control groups respectively. The food diary response rate to the official NDNS was 56% (Bates et al., 2014).

Due to the fact that UK nutritional requirements are grouped into two age brackets that did not exactly match the participant age group of this study, it was decided to use the 7-10 year age bracket for comparison (Dept. of Health, 1991). Using this age bracket as a guide, participants met the Estimated Average Requirement (EAR) for all nutrients. Intakes of some minerals appeared suboptimal (iron 72% of RNI, zinc and magnesium both 74% of RNI), however all were above the LRNI. This is remarkably similar to data for the most recent NDNS

report, which reported that mean intakes of all minerals were close to or above the RNI for children aged under 11 years and few children in this age group had intakes below the LRNI (Bates et al., 2014). The finding that boys had significantly higher intakes than girls for protein, sodium, iron, zinc, magnesium, iodine and phosphate is also reflected in national statistics, which found girls had lower intakes of minerals specifically iron, zinc, iodine and calcium. Median vitamin D intakes were low in all participants (1.83 mcg/day). Mean daily intake of vitamin D for 4-10 year olds and 11-18 year olds in the most recent NDNS was 2.7mcg and 2.4 mcg respectively, with 20% of children having low serum vitamin D concentrations (Bates et al., 2014). Although there is no DRV in the UK for vitamin D for children over five years old, using the arbitrary amount of 10mcg/day (Meyer et al., 2015); it can be concluded that intakes in this group of participants are highly deficient.

Although we did not specifically quantify the amount of milk and dairy products consumed by participants, it could be speculated that those in the CME group consume less of this food category and therefore parents give dietary supplements to compensate for the possible deficit incurred. Dairy products are an important dietary source of most micronutrients, including calcium, phosphorus, magnesium, zinc, iodine, potassium, vitamin A, vitamin D, vitamin B12, and riboflavin (vitamin B2). In the UK, milk consumption has decreased overall by 8.1% in 10 years, although admittedly this statistic reflects household consumption, rather than children's intakes specifically (DEFRA Family Food Survey 2012). Looking at individual childhood consumption in the UK, mean daily intake of whole milk is 86ml and 33ml for 4-10 and 11-18 year olds respectively, semi skimmed milk intake is 105ml and 100ml respectively, mean daily cheese intake is 10g and 11g respectively and mean daily yoghurt intake is 39g and 19g respectively (Bates et al., 2014). This translates in household measures to a cup of milk, one tablespoon of grated cheese and approximately ¼ a standard size pot of yoghurt per day; which added together appears to be quite a small amount. Data suggests that dairy product consumption by children and adolescents in many countries has declined (Dror & Allen, 2014). In the USA, a nationwide study of young people reported that 34.2% of 7557 participants were classified as "non milk drinkers", consuming less than a quarter cup of milk per day (Murphy, Douglass, Johnson, & Spence, 2008). Intakes of calcium, phosphorous, magnesium, potassium and vitamin A were found to be significantly lower in non-milk drinkers in every age category, however the study did not take into account other sources of dairy products (i.e. cheese, yoghurt).

As this study is the first identified from the literature to assess nutritional intake in individuals who *previously* consumed a CME diet, but now consume an unrestricted diet, there

is a lack of literature to compare directly against. As had previously been discussed, there are a number of studies that have investigated dietary differences in children whilst consuming exclusion diets at various ages, but it is difficult to draw parallels, as the participant inclusion criteria for the experimental group in those studies is the opposite to this study. In this study, the significantly lower intakes for iodine ($p < 0.01$) and riboflavin ($p = 0.029$) observed in the CME group could be attributed to a lower intake of dairy products. In the NDNS, the major contributor to riboflavin intake was 'milk and milk products', accounting for 41% of daily intake in for children aged 4-10 years old. Similarly 'milk and milk products' was the largest contributor to iodine providing 51% to children aged 4-10 years (Bates et al. 2014). In support of this, an Australian study of 4487 2-16 year olds found that drinking milk was associated with higher a total micronutrient intake, specifically iodine intake was nearly double in milk drinkers (Fayet, Ridges, Wright, & Petocz, 2013).

Conversely, the significantly higher intakes in the CME group for sodium ($p = 0.018$) and selenium ($p = 0.016$) could be explained by proportionately higher intakes of non-dairy foods in these children. NDNS data indicates that approximately one third of both sodium and selenium intakes in 4-10 year olds is derived by cereal products, followed by meat and meat products as the next highest contributor (Bates et al., 2014). The higher, although non significant, intakes of calcium, iron, zinc, magnesium, phosphorous, thiamine and vitamin B12 by the control group, would also fit with this hypothesis as these micronutrients are all found in useful concentrations in dairy products, with the exception of iron. Likewise the non-significant trend of higher intakes of fibre, vitamin A, vitamin C and vitamin E in the CME group, would comply with this hypothesis as these are nutrients that are typically found in higher in fruit, vegetables and plant-based fats. Indeed it has previously been suggested that children with a history of food allergy have a tendency to establish "healthier" eating habits, such as less meat and more vegetables (Mukaida et al., 2010). Overall it is unlikely that any of these nutritional differences between groups would have a meaningful health significance as both groups met the EAR for all nutrients. However, the suboptimal vitamin D content across all participants is of concern.

The finding that 20.7% of all participants took daily dietary supplements daily is higher than the 8-16% rate reported in 4-18 year olds in the NDNS (Bates et al., 2014). Our finding that 25% of the CME group took dietary supplements is slightly lower than the 30% rate reported by Meyer et al. (Meyer et al., 2015), however their population included children consuming multiple exclusion diets. Interestingly, Meyer et al. (Meyer et al., 2015) reported that the use of supplements meant intakes of some micronutrients (vitamin B6, folate, vitamin C,

vitamin B6, thiamine, selenium and vitamin A) exceeded 200% of the recommended amounts. Similarly we found that children who took dietary supplements had a significantly higher vitamin B6 ($p < 0.01$), folate ($p = 0.01$) and vitamin C ($p = 0.037$) intakes than those who did not, however as these are water-soluble vitamins that are not stored in the body, there is little risk of toxicity.

5.6.9 Taste Preference

Taste preference was set as an exploratory objective for this study as it was thought there would be insufficient numbers of participants to successfully power this objective, particularly to compare different sub categories of infant formula within the CME group. However despite this, the trend approaching significance that bitter taste was better preferred by the CME group is an important finding. It is supported by previous studies and concurs with the hypothesis that feeding infants altered tasting hydrolysed and soya formulae can manipulate taste preferences to like innately disliked sour and bitter tastes (Mennella & Beauchamp, 2002). The lack of correlation between any taste preference and any growth measurement or infant feeding variable, number of symptoms or number of food excluded is not surprising given the limited sample size.

The only identified study in the literature that assessed taste preference in children previously fed substitute formula and currently older than seven years old, is that of Sausenthaler et al. (2010). This large scale study of 833 ten year old children in Germany found a positive association between feeding with any kind of hydrolysed formula in infancy and the acceptance of extensively hydrolysed casein formula at age 10 years; although the data distribution was extremely skewed because all children in the study rated the taste of the formula very negatively (Sausenthaler et al., 2010). To a certain extent, testing preference to infant formula in children aged 10 years old is of little practical relevance as it is not something children of this age currently consumed or would ever consume in the future. Sausenthaler et al.'s study did not include a group who were breastfed or fed with either an AAF or soya formula; rather groups who were fed either cows' milk formula, EH casein formula, EH whey formula or a partially hydrolysed whey formula. Therefore the results are difficult to compare directly to the present study.

It could also be argued that due to the proportion of children fed with soya formula in the present study and the fact that it is no longer recommended as first line treatment for infants with CMA under six months (Fiocchi et al., 2010; Koletzko et al., 2012; NICE, 2011; Venter, Brown, Shah, Walsh, & Fox, 2013), the results are not of practical relevance or generalisable

to the current management of CMA. However, we did not detect any difference between formula groups for bitter taste preference, therefore it is not possible to say whether being fed a whey or casein EH formula, an AAF or a soya formula has any greater effect on bitter taste preference. Additionally amongst the CME group, because bitter taste preference was not found to be significantly correlated with age of introduction/duration of substitute formula, age of introduction of solids, duration of breastfeeding or number of foods excluded, it is difficult to draw any firm conclusion on these infant feeding factors. The finding that bitter taste preference was positively correlated with "enjoyment of food" (rho = 0.450, p = 0.027) within the CME group is intriguing as it suggests that children with liking for bitter foods are more likely to gain pleasure from meals, but this finding was not supported by an association with any other measure of fussy eating, food neophobia, or food preference category.

The taste preference methodology used, although basic and simple in approach and exploratory in nature, used validated scales and dilution of taste substrates that have previously been identified as appropriate in this age group (Knof et al., 2011; Kroll, 1990). Perhaps the use of real food rather than flavoured water would have provided more meaningful and "real world" implications, however as previously discussed the conduct of sensory research in children is complex and labour intensive (Popper & Kroll, 2005). Participation in the taste preference test was high, implying excellent acceptability in this age group. All of those who attended the research appointment took part; only one participant did not take part due to time constraints. The other four participants for whom data was not available completed the questionnaires by post and therefore were not available to complete the taste preference test in person.

5.6.10 Growth

The lack of significant difference detected between dietary exclusion groups is to be expected given the small sample size, the multitude of factors that influence growth in children and the fact that most macro and micro nutrient intakes did not differ significantly between groups**.** As previously discussed, growth of children with CMA and other food allergens has been thoroughly investigated across many different countries and age groups (Agostoni et al., 2007; Flammarion et al., 2011; Isolauri, Sutas, Salo, Isosomppi, & Kaila, 1998; Mehta, Groetch, & Wang, 2013; Meyer et al., 2014; Seppo et al., 2005; Vieira et al., 2010). The only study comparing long term growth of children fed substitute formula for CMA did not show any difference in growth at age 10 years, however children in this study were only fed the substitute formulas for a period of 16 weeks (n = 926) (Rzehak et al., 2009). A Japanese study of 7-15

year olds (n = 14669) (Mukaida et al., 2010) reported that those with a history of following an exclusion diet for any food allergy had lower weight z scores, with an overall lower incidence of overweight and obesity; however the data on food avoidance was collected retrospectively.

With the exception of waist circumference, which was very high in both groups, all other growth measurements can be considered relatively normal. The high median waist circumference centile (98.8%) is unexpected. It is possibly a reflection of the rising rate of central obesity and the fact that the waist circumference charts used as comparison are now outdated, having been derived from data collected in 1990 (McCarthy, Jarrett, Crawley & McCarthy, 2001). The overall percentage of children classified as overweight or obese (19%) is lower than national statistics, with the most recent National Child Measurement Programme data for 2013/14, showing that 19.1% of children aged 10-11 were obese and a further 14.4% were overweight (Health and Social Care Information Centre, 2014). However it is particularly interesting that proportionately nearly double the amount of children in the CME group (28.6%) were classified as overweight/obese compared to the control group (15%). Meyer et al. (2014) has previously identified that obesity is an increasing concern in children with food allergy and that the emphasis should not always be on under nutrition.

Even though the difference between groups was not statistically different, the finding is very thought-provoking. Although the data is not consistent, an increasing body of research suggests there is a strong association between consuming dairy products in childhood and reduced risk of overweight/obesity in late life. A longitudinal study of children (n = 99) in the USA reported that those with the lowest intake of dairy products between 3-6 years of age had significantly greater gains in body fat during by early adolescence (10-13 years old) (Moore, Bradlee, Gao, & Singer, 2006). Likewise an Italian study of 884 children, of mean age seven years, highlighted that the risk of overweight was significantly higher in those with lower whole milk consumption, which was independent of age, birth weight, parental overweight, education, physical activity and other dietary habits; which may be due to the unique satiating effect of milk (Barba & Troiano, 2005). It has been reported that those who avoid milk consume more sweetened beverages (DeBoer, Agard, & Scharf, 2015), which could also contribute to a higher caloric intake. As we did not specifically quantify dairy food intake, sweetened beverage intake and did not measure body composition, it is not possible to know the reason for the increased number of overweight and obese children in the CME category. We also did not record or account for physical activity or sedentary behaviour. However it is clearly an area that requires further examination in future research studies.

5.6.11 Overall strengths and limitations of study

5.6.11.1 Strengths

This study has a number of key strengths. Firstly the infant feeding data was collected prospectively for 95% of participants and has been previously published in highly ranked allergy journals (Grimshaw et al., 2013, 2014; Venter et al., 2008; Venter et al., 2009). For the minority of participants (n = 5) that were not recruited from the two birth cohort studies, data was validated against medical and dietetic notes. Many studies in the area of infant feeding and later health employ a retrospective study design, which limits the validity of their findings (Galloway et al., 2003; Shim et al., 2011; Townsend & Pitchford, 2012). The CME and control groups were closely matched for demographic variables and appropriate statistical techniques were used to control for covariates where appropriate. The questionnaires used were previously validated and known to be suitable for the age group. The study employed a combination of parent and child report measures to explore the primary objectives from both perspectives. No participant had difficulty completing the questionnaires and the methods were clearly understood. Children were capable of undertaking the taste preference test with ease and enjoyed the process. All measurements, questionnaires and analysis were undertaken by the same researcher to minimise bias, with coding and data entry checked to detect any errors.

5.6.11.2 Limitations

There were also a number of limitations to this study that need to be acknowledged so that the results can be interpreted with due caution. Firstly it is possible that a recruitment bias exists, with those who were more interested in food allergy, health and diet more likely to take part. However previous follow up studies of the FAIR cohort has demonstrated an excellent retention rate of participants, suggesting a recruitment bias is not at play (Venter, Patil, et al., 2015). The two birth cohort studies (FAIR and PIFA) took place five years apart, involving two separate research teams, during which time the infant feeding guidelines changed, with regard to both introduction of solid foods and use of soya formula for management of CMA. This means the CME children from both cohorts may have received slightly different dietary advice and management. The study protocols for both studies and the exact type of data collected, although not too dissimilar, had slight differences (Grimshaw et al., 2014; Venter et al., 2008). However both birth cohorts took place in the same region of the country and by incorporating both studies, allowed a broader age range and greater number of children to be recruited.

The lack of ethnic diversity and higher educational level of study participants means the study is not necessarily generalisable to other populations, however as previously discussed

the ethnic background it is reflective of the local population of Hampshire and Isle of Wight. Previous studies from around the world have shown that children's food and taste preferences and levels of fussy eating are consistent across cultures therefore regional and country specific food habits may not be of great importance.

Unfortunately despite best efforts with recruitment the study was not sufficiently powered. Forty-three participants were required in the CME group, however only 28 were recruited. Only a finite number of participants who met the inclusion criteria for the CME group existed across the two birth cohort studies, therefore this is why an additional five participants were recruited using retrospective NHS clinic records. Several attempts were made to contact and recruit additional CME participants from the FAIR study and clinic records. It was not possible to recruit further CME participants from the PIFA study due to time constraints. The lack of power could explain why some differences were not observed between groups. However, despite not being adequately powered, the recruited number of 28 in the CME group and 101 participants in total compares very favourably to other studies of this kind. Although food preference and growth studies typically recruit large number of participants (> 300), other published studies investigating fussy eating, taste preferences, nutritional intake and food neophobia have recruited far less (Tiainen et al. (1995) (n = 38); Henriksen et al. (Henriksen et al., 2000) (n = 34); Flammarion et al. (2011) (n = 201); Mennella & Beauchamp (2002) (n = 83); Pliner & Pelchat (1986)(n = 55); Skinner et al. 2000 (n= 70)).

5.7 Conclusion

Overall this study of 101 children aged 7-13 years old from the South of England demonstrated that consuming a CME diet during infancy has an effect on some, but not all, eating habits in later childhood that were under investigation. Children who were fed a CME diet during infancy were found to have significantly higher levels of avoidant eating behaviour, to be slower eaters, to have lower liking scores for some dairy products and to have different intakes of some micronutrients up to ten years after cows' milk had been reintroduced into the diet. There were non-significant trends for children in the CME group to prefer bitter tastes and to have a lower consumption of some micronutrients. A greater proportion of children in the CME group were classified as overweight/obese category. Fussy eating in early life was a predictor of fussy eating in later childhood in all participants. No significant differences were found between groups for liking of any food category, food neophobia, macronutrient intake or growth measurements. All participants were found to have suboptimal intakes of vitamin D and higher than average waist circumferences.

In general, the data reported from this study is largely in agreement with the existing literature. Additionally some interesting novel findings have been made. It can be concluded that consuming a CME diet for CMA during infancy is associated with changes to eating behaviour that persist approximately ten years after cows' milk has been reintroduced into the diet. However given that the avoidance of cows' milk in the short term is currently the only management option for resolution of symptoms in CMA, this will not fundamentally change the management of CMA in routine clinical practice. The implications of these findings are that problem eating habits in children consuming an exclusion diet for CMA need to be addressed early and that reintroduction of cows' milk containing foods needs to be adequately and timely monitored to ensure that negative long term outcomes do not occur.

6 Chapter six: General discussion & conclusion

6.1 Chapter overview

This chapter brings together the overall findings of the two studies of this PhD, starting with a brief recap of the rationale and aims of the research. The main results of both studies are summarised and the implications of the research are explained. The chapter is concluded by addressing methodological strengths and limitations and outlining future research needs.

6.2 General discussion of findings

6.2.1 Overview of aims and hypothesis of thesis

This study aimed to find out if consuming a CME diet and/or substitute formula in infancy for the management of CMA has any short or long terms effects on eating habits and behaviour. The rationale for this being that to date there is a paucity of research investigating the prevalence of eating problems in children consuming exclusion diets for CMA (Haas, 2010). Although anecdotally there appears to be higher levels of fussy eating and feeding difficulties and lower dietary variety in children with food allergies, this had not been conclusively demonstrated to be the case. Studies that have partially investigated this issue have not always used a control group and generally been limited to children with the most severe presentations of food allergy (Meyer, Rommel, et al., 2014; Mukkada et al., 2010; Wu et al., 2012). Despite the fact that substitute formula have been used for several decades in the management of CMA and are known to affect taste preferences in the short term (Beauchamp & Mennella, 2009; Liem & Mennella, 2002), to date there had not been any studies examining the long term effect of consuming a CME diet and substitute formula on food preferences and fussy eating in children.

The hypothesis therefore was that children who are consuming a CME diet for CMA would have altered eating behaviours at the time of the exclusion diet and several years after cows' milk was reintroduced into the diet. In order to evaluate this hypothesis, two separate cross sectional studies were undertaken, recruiting children from NHS allergy clinics, health visitor clinics and birth cohort studies on the Isle of Wight and Hampshire in the South of England. Overall it was shown that children consuming a CME diet have higher levels of some negative eating behaviours; specifically higher levels of fussy eating, feeding difficulties and food neophobia at the time of the exclusion diet and higher levels of avoidant eating behaviour alongside reduced preference for some dairy foods in the long term. Because the research

design consisted of two separate cross sectional studies, rather than a longitudinal or randomised control study design, it is not possible to demonstrate cause and effect, only association. However the research design, using a control group in both studies and validated methods appropriate to the age groups, was robust. Confounding variables and biases were carefully considered throughout recruitment, data collection and analysis.

6.2.2 Findings in relation to eating behaviours in healthy children

Although this thesis focused on eating behaviours in food allergy and recruited children consuming an exclusion diet for CMA, the findings relate and extrapolate to food practices in infants and children in general. As previously discussed in detail, early infancy is a plastic time in development, therefore manipulation of the diet and exposure to different foods has inevitable consequences. Problematic eating habits occur commonly in healthy children, as do conditions such as colic, gastro-oesophageal reflux and constipation; all of which are usually transient.

Infants and toddlers in the control group of study one frequently reported symptoms of vomiting and colic, reporting on average two symptoms compared to four symptoms in the dietary exclusion group. Because fussy eating levels were not significantly different between challenge proven and challenge negative children in the CME group, it may be the presence of symptoms per se, rather than a finite diagnosis of food allergy that could increase levels of fussy eating. If this is the case, then the presence of non-allergic gastro-oesophageal reflux, colic and constipation are also likely to affect eating behaviours in children without food allergy. This has implications for the high proportion of healthy children who present with these frequently occurring problems of early childhood. It also concurs with the assertion of Bergmann et al. (2014) that these functional gastro intestinal symptoms are very common and should not necessarily lead to prescription of an exclusion diet unless the clinical history is strongly indicative of CMA.

Individual patterns of food preferences and eating behaviour will develop based on the foods offered and context of feeding experiences. It has been shown that in preschool children the most limiting factor in their preferences are the foods never offered to them (Carruth et al., 1998). Therefore ensuring all children are exposed to a wide variety of age-appropriate foods in early life is paramount to preventing fussy eating in the first place. Associations were shown in this study between fussy eating in early childhood and higher levels of avoidant eating behaviour at ages 7-13, which was a trend seen in both control and dietary exclusion groups. The significantly lower scores observed for “enjoyment of food” and lower preference scores

for many food categories in those reported to be fussy eaters at 1-2 years old and 2-3 years is striking. It underlines the importance of promoting a suitable eating environment in early childhood, ensuring positive parental reinforcement of dietary practices in all children. Although this particular data was collected retrospectively and could be subject to recall bias, it concurs with previous prospective research demonstrating definite tracking of eating behaviour traits (Ashcroft et al., 2008; Cardona Cano et al., 2015; Mascola et al., 2010).

6.2.3 Findings in relation to other medical conditions

Several paediatric medical conditions require dietary manipulation, some of which also require a substitute formula for a certain amount of time (e.g. faltering growth, cystic fibrosis, inborn errors of metabolism). Although all of these medical conditions are highly complex and obviously very different to food allergy in their pathophysiology, natural history, duration and presentation, some commonalities in their dietary management can be drawn.

This study indicated that *excluding* a food group in early life had an effect on preference for that food up to ten years later. In this study it was relatively high fat/sugar foods that are typically perceived as 'unhealthy' that were disliked (e.g. chocolate, ice cream, butter). It is arguable that the converse could also be true (i.e. promotion and deliberate *inclusion* of certain foods in early childhood will lead to increased preference in later life). This has relevance for faltering growth and infants born small for gestational age, conditions in which a substitute formula and high energy foods may be promoted, albeit temporarily for a finite period of time. Therefore this study adds weight to the argument that manipulation of dietary intake in children in infancy can have profound effects on diet in later life.

6.2.4 Findings in relation to management of fussy eating, food neophobia and feeding difficulties

Fussy eating, food neophobia and feeding difficulties are commonplace. Although dismissed as benign and pervasive, many parents seek health professional advice so knowing more about the predictors and consequences of these behaviours will potentially inform health professionals of the scale of the problem enabling them to manage these problems better. Indeed the finding that those with the highest levels of feeding difficulties and food neopohobia had the most dietetic contact highlights the impact on clinical resources. Of note, there was no difference in dietetic contact time whether the participant had challenge proven CMA or not.

It must be reiterated that although significantly higher levels of fussy eating, food neophobia and feeding difficulties were observed in the CME group in the infant/toddler study, these levels were all within 'normal' levels. The prevalence of feeding difficulties in the CME

group was 13.6% compared to 1.6% in the control group. This is considerably lower than previous estimates of feeding difficulties in healthy children from other studies (Benjasuwantep et al., 2013; Crist & Napier-Phillips, 2001; Lewinsohn et al., 2005; Wright et al., 2007), underlining the significance of using a validated questionnaire. The fact that feeding difficulty score was significantly higher in participants with maternal food allergy history implies that mothers with food allergies are more likely to report feeding problems in their child, which may or may not be a true reflection of the problem. Being aware of these issues is important for health professionals working with children with food allergy. Overall the results emphasise that most children with CMA do not have levels of eating problems that prompt overt concern, however conducting a thorough nutritional assessment and differentiating between feeding difficulties and feeding *disorders* is critical (Kerzner et al., 2015). This is said with the caveat that the children recruited for this study were from a secondary care allergy clinic. Children who have multiple food allergies or those seen in tertiary care clinics may have more severe eating problems.

6.2.5 Findings in relation to management of cows' milk allergy

Several international and national guidelines emphasise the importance of taking a thorough clinical history and/or appropriate allergy tests to ensure an accurate diagnosis (Boyce et al., 2010; Fiocchi et al., 2010; Luyt et al., 2014; NICE, 2011). Without an accurate diagnosis, exclusion diets will be consumed unnecessarily or symptoms will persist untreated. Although the aim of this study was not concerned with rates of mislabelled CMA or unnecessary exclusion diets, its findings are undeniably interrelated.

The finding that avoidant eating behaviour is higher in children aged 7-13 years old who consumed a CME diet during infancy is both novel and concerning. It demonstrates a persistent negative effect of consuming a CME diet up to ten years after cows' milk products (should) have been reintroduced into the diet. It emphasises the need to ensure that the diagnosis of CMA is correct, as secondary effects are apparent several years later. It underlines the importance of challenging infants after a 2-4 weeks *trial* of a substitute formula if mild to moderate non-IgE CMA is suspected (Venter et al., 2013), rather than initiating a CME diet and leaving the infant consuming a CME diet unchallenged for an indeterminate amount of time. The high usage of AAF in this study perhaps indicates that substitute formulas were not being appropriately used in all cases. Therefore when prescribing CME diets, health professionals in both primary and secondary care need to ensure the diagnosis is correct, use the most appropriate substitute formula and look longer term, beyond the immediate exclusion diet.

Following on from this, the finding that children consuming CME diets have a less varied diet is not revelatory in itself. However with the recent emphasis on dietary diversity and allergic outcomes (Grimshaw et al., 2014; Nwaru et al., 2014; Roduit et al., 2014), it underlines the importance of incorporating substitute products into the diet adequately, suggesting more milk-free alternatives and helping parents to adapt meals and recipes accordingly. The lower variety in the 'meat/fish' category perhaps suggests that some foods may be over restricted and that dietary advice could be improved to help expand the diet, as well as emphasising the ubiquity of cows' milk in all foods. On a positive note, the finding that children adhering to a CME diet consume less sweet/fatty foods, less non-water drinks, their parents are more aware of healthy eating and their growth status is equivalent to children in the control group is likely to be a positive testament to the dietetic input and advice they have received.

The lower preference for chocolate, ice cream, butter and full fat milk in the children who consumed a CME diet during infancy may be seen as inconsequential or indeed a beneficial finding, considering these foods are high in fat and/or sugar. Although food preference is predictive of intake in children (Birch, 1979), it is what is eaten, not what is liked that is fundamentally of importance. However it must be emphasised that nutritional differences were also found between the CME group and the control group in certain micronutrients, which is a novel finding. Dietary research regarding CMA often focuses on calcium and vitamin D, however the micronutrients that were significantly lower in the CME group (riboflavin and iodine) are also both prominent in dairy foods. The absence of a difference between groups for calcium intake is probably because there is an awareness of calcium supplementation amongst parents. This may however mean that other micronutrients may be overlooked, demonstrating the importance of individualised nutritional assessment and supplementation where appropriate (Meyer et al., 2015). In saying that, the finding that both groups had very low vitamin D intakes also warrants attention.

6.3 Future research needs

6.3.1 Other food allergens

Although this study demonstrated a number of novel findings in relation to CMA and eating behaviour, by definition it only examined the exclusion of cows' milk and use of substitute formula. Allergy may occur to a number of different foods at various stages in life. It is not known whether the patterns of behaviour found in this study extends to the exclusion of other foods. As explained earlier, the rationale for examining CME diets is that milk is the first and predominant infant food for some months. The exclusion of other foods is arguably of less

importance from a development of food preference and nutritional perspective. However, on the other hand, other food allergens (e.g. peanut or egg) are widely dispersed in food products, the duration of allergy can be prolonged in comparison to CMA and adverse reactions to these foods can be severe. The most important finding in relation to other food allergens is the dislike of the previously excluded food and risk of non-inclusion in the diet once tolerance is achieved. This is particularly relevant if a food (e.g. peanut) is not consumed in the diet following a negative challenge. Therefore future research should explore in more detail the extent to which other food allergens are disliked, the extent of their reintroduction and the risk of recurrence of food allergy once tolerance is initially achieved.

6.3.2 Longer duration of follow up

The findings in relation to avoidant eating behaviour and dislike of some dairy products were evident in a group of children at age 7-13 years. However it is not possible to say whether this effect persists into adulthood. It may be if the participants were followed up later in adolescence or into early adulthood, greater exposure and familiarity would occur, meaning preference and fussy eating would improve. There is disagreement when precisely the “window of opportunity” occurs for optimal development of food preference and whether there is more than one “window of opportunity”, with some authors citing learning about foods as a continual process (Szczesniak, 2002). Therefore future research could undertake a longer period of follow up to examine whether avoidant eating behaviour and food preferences in children with previous food allergy are permanent or adaptable. Measurement of genetic taste preference should also ideally be included as part of such a study.

6.3.3 Larger sample size with sub group analysis

Finally and most importantly, future research involving a larger sample size, including more participants in each of the substitute formula groups would enable more detailed statistical analysis to be undertaken. It was not possible to say with the number of participants recruited in the current study whether there were differences between substitute formula groups. It is known that each substitute formula has a different taste profile and a different composition, therefore it may be that food and taste preference outcomes would be different depending which formula is consumed. Randomisation to a specific formula group would be a more stringent study design, however this would be ethically difficult due to the varying spectrum of symptoms that occur in CMA and the need to encourage breastfeeding. Future research should also aim to include a separate group of breastfed infants to explore the impact of maternal CME

diet on food preferences and habits. This would also allow the effect of the CME diet and the substitute formula to be assessed separately.

6.4 Methodological considerations and overall conclusion

6.4.1 Limitations

As previously mentioned, the major limitations of this research are that participants in study one did not have food challenges to conclusively confirm the diagnosis of CMA and secondly, that not enough participants were recruited for the CME group in study two. These limitations are justified by the fact that food challenges for CMA are not generally undertaken in infants in secondary care, usually a doctor diagnosis and a clinical history are sufficient. Secondly, it was not feasible; despite best efforts to recruit any more participants for the CME group in study two within the given timeframe. Fundamentally these limitations mean that it is not possible to disentangle the effect of the substitute formula, the CME diet and the allergic symptoms on the outcome measures. However in practical terms, this may not be overly important. As the vast majority of infants with CMA are not exclusively breastfed, a CME diet and a substitute formula will nearly always be consumed simultaneously.

Methodological issues concerning the collection of dietary data have already been described in detail and are inherent in any study that assesses nutritional intake. The most appropriate dietary assessment methods were chosen for each study (FFQ and food diary) and attempts were made to minimise biases during data collection and analysis. It was not possible to measure all of the factors that impact on childhood eating habits and growth, such as genetic taste preference, physical activity or parental eating habits. However these factors are acknowledged and there is no reason to believe they would necessarily be any different between dietary exclusion and control groups. It is also of course acknowledged that both studies were cross-sectional, therefore the findings are associative rather than cause and effect, however confounding variables have been controlled for in data analysis.

6.4.2 Strengths

The study design of two cross sectional studies allowed data to be collected at two important age ranges: infancy, where eating behaviours start to develop and later childhood, where independence and autonomy is growing as children enter adolescence. A thorough literature search was undertaken to determine the factors most associated with the outcome measures, in order to select the most suitable tools and measurements. Therefore both studies used validated and age appropriate methods, which is fundamental, especially when there has been

little consistency between studies in previous research. Recruitment of participants from two birth cohort studies meant the majority of infant feeding data was prospectively collected, thus limiting recall bias. The control and exclusion groups were well matched for nearly all sociodemographic variables and were reflective of the population from where they were recruited. Data was collected, recorded and analysed by the same researcher to minimise researcher bias.

6.5 Overall conclusion

The overall conclusion of this research is that health professionals and parents should consider the wider implications beyond the exclusion diet in the management of CMA. Excluding cows' milk is essential for symptomatic relief in CMA and use of substitute formula is necessary in those not breastfed. However, given that levels of fussy eating, feeding difficulties and food neophobia are higher in those consuming a CME diet, attention should be paid to ensure that dietary variety is optimised as much as possible and eating problems are managed positively and appropriately. In saying that, in the majority of children the outcome eating behaviour measures were within normal levels and growth was not affected Negative effects of the exclusion diet are however apparent several years after it has ended, demonstrating the powerful effect that early infant feeding has on later childhood.

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Appendix 28: Recruitment letter for NHS clinic participants

Appendix 29: UPR16 form

Appendix 1

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Title Page

Title "Cows' milk exclusion diet during infancy: Is there a long term effect on children's eating behaviour and food preferences?"

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Abstract page

Cows' milk exclusion diet during infancy: Is there a long-term effect on children's eating behaviour and food preferences?

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Background: Dietary restriction during infancy may influence later eating behaviour. The aim of this study was to determine if consuming a cows' milk exclusion (CME) diet during infancy affects eating habits in later childhood, once cows' milk has been reintroduced into the diet.

Methods: Children were recruited from two large birth cohort studies in the UK. A small number of participants were recruited from allergy clinic. Two groups were recruited: an experimental group of children who had consumed a CME diet during infancy and a control group, who had consumed an unrestricted diet during infancy. Parents and children completed questionnaires regarding eating behaviour and food preferences.

Results: 101 children of mean age 11.5 years were recruited (28 CME and 73 control). The CME group scored significantly higher on "slowness of eating" and on the combined "avoidant eating behaviour" construct ($p < 0.01$). The number of foods avoided and symptoms were associated with higher levels of avoidant eating behaviour ($p < 0.05$). The CME group rated liking for several dairy foods (butter, cream, chocolate, full fat milk and ice cream) significantly lower than the control group ($p < 0.05$), although there were no significant differences seen for any other category of food.

Conclusion: This study demonstrated that consuming a CME diet during infancy has persistent and long-term effects on eating habits and food preferences. To reduce future negative eating behaviours, children's exclusion diets need to be as varied as possible and reintroduction of cows' milk products closely monitored.

Key words: Cows Milk Allergy, Eating Behaviour, Fussy Eating, Infant Diet.

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Introduction

Cows' milk allergy (CMA) affects between 1.26-2.8% of young children in the United Kingdom (UK) (1,2), although self reported levels of CMA are much higher (3). In the majority of children, CMA will resolve by age two years, when cows' milk products can

successfully be tolerated(1,2), although severe phenotypes exist with persisting CMA into older childhood (4,5). The usual natural history of CMA therefore provides an opportunity to explore the effect of dietary exclusion in infancy on later dietary outcomes. Breast or formula milk is the sole source of nutrition in the first few months of life and remains the major source of nutrition for some time after the introduction of solid food. Cows' milk is therefore a unique allergen, in that it dominates the early nutrition needs of infants, whether via infant formula or transmitted from the maternal diet via breast milk. Its exclusion has arguably more impact on nutrition and eating habits than the exclusion of other food allergens.

We have recently reported that infants and toddlers consuming a cows' milk exclusion (CME) diet have significantly higher scores for fussy eating and feeding difficulties compared to children consuming an unrestricted diet (6). Whether these differences are persistent over time is unknown. Several factors suggest that consuming a CME diet in infancy may affect eating behaviour in later life. Firstly, it is known that innate taste preferences in early infancy (i.e. liking of sweet and rejection of bitter) can be manipulated by exposure to the altered taste of substitute formula used for management of CMA. These formulae have been shown to affect preference for savoury, sour and bitter foods in infancy (7) and up to the age of 4-5 years of age (8). Secondly, it has been shown that the number and type of certain allergic symptoms such as wheeze, colic, vomiting and diarrhoea are associated with negative eating behaviours (6,9,10). Whether the negative eating behaviours persist once the symptoms resolve is unknown. Finally, it is known that a proportion of food allergic children never reintroduce the culprit food allergen into their diet following a negative oral food challenge (11)(12). This has potential to influence dietary intake if the food(s) is ubiquitous and nutrient dense.

The influence of these factors on eating behaviour, growth and nutritional intake is unclear. To date, no research has evaluated if there is a long-term impact of avoiding cows' milk in early infancy on food preferences and eating behaviours in later life. Therefore the overall aim of this study was to determine if following a CME diet during infancy affects eating habits in later childhood, once cows' milk has been reintroduced into the diet.

Methods

Study Design

This was a cross sectional study of 7-13 year old children from the Isle of Wight and Winchester area, UK. The study design is shown in Figure 1.

This study had two groups: a CME group and a control group. Children were eligible for inclusion in the CME group if they had consumed a substitute formula and/or a CME diet in the first year of life for ≥ 3 months. Children excluding other foods, in addition to cows' milk were also eligible for inclusion. Participants were primarily recruited from two birth cohort studies; the Food Allergy and Intolerance Research (FAIR) and Prevalence of Infant Food Allergy (PIFA) studies. The FAIR study recruited infants born in 2001/2002 from the Isle of Wight (1). The PIFA study recruited infants born in 2006-2008 from the Winchester area (13). A small number of participants were recruited from NHS allergy clinics from the Isle of Wight.

The control group, also recruited from the FAIR and PIFA studies, was composed of children who had consumed an unrestricted diet during infancy. Children with current food allergy or any medical conditions requiring a special diet were excluded.

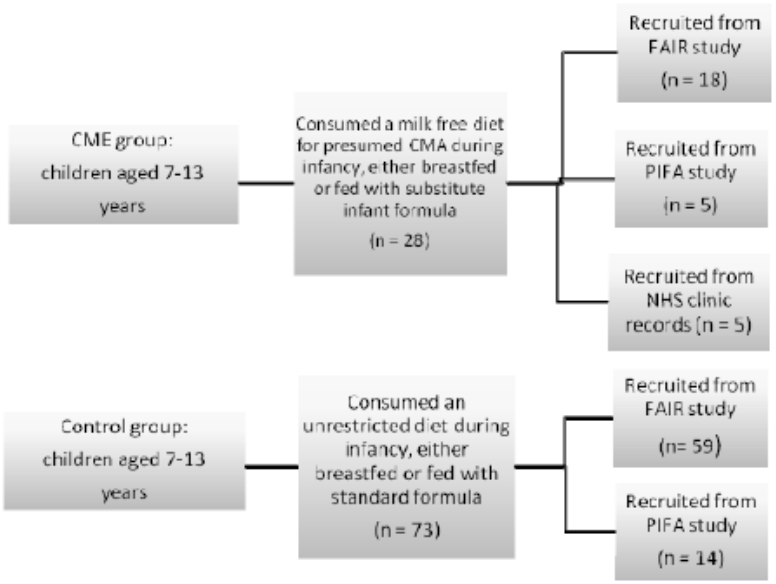

Figure 1. Study design and numbers recruited.

Data collection

Data collection took place between July 2013 and April 2015.

The Child Eating Behaviour Questionnaire (CEBQ) (14) was completed by parents. It consists of four subscales that measure one positive aspect ("enjoyment of food") and three negative aspects of eating behaviour ("fussy eating", "food responsiveness" and "slowness in eating"). Items are measured using a five-point scale.

Children's food preferences were assessed using a questionnaire consisting of 115 common food and drink items categorised into nine groups (15). Children were asked to choose between six options to indicate "how much you like each food".

Extensive information about social demographics, infant feeding, family and allergy history was available from the original birth cohort dataset. For participants recruited from NHS allergy clinics, this information was extracted from medical notes.

The study was approved by Berkshire NHS ethics committee (reference 13/SC/0194).

Data analysis

Questionnaires were scored according to published guidelines by the original authors. Data was analysed using SPSS software (IBM, version 20). Descriptive statistics were calculated. Differences between the CME and control groups were compared using Mann Whitney or chi square tests. Analysis of Covariance (ANCOVA) tests were undertaken to compare groups whilst controlling for covariates. Spearmann rho correlations were performed. The significance level was set at 0.05 for all analyses. A power calculation for a two tailed outcome, at 80% power and at a significance level of 0.05 indicated that 129 participants were required for this study. Sample size calculations were made on the basis of a detecting a 15% difference in food preference category scores with a ratio of 1:2 CME group: control group.

Results

Description of participants

101 participants were recruited, 28 in the CME group and 73 in the control group. The proportion of participants recruited from the FAIR study, PIFA study and NHS allergy clinics is shown in Figure 1. Demographic characteristics of participants are detailed in Table 1. There was no differences between groups for any of these variables, except maternal and sibling food allergy.

Table 1. Demographic characteristics of participants overall and by dietary exclusion group.

*significant difference between CME and control group $p < 0.05$

	All (n =101)	CME group (n =28)	Control group (n = 73)
Median age in years	11.5	11.33	11.58
(minimum-maximum)	(7.04 – 13.83)	(7.25 – 13.83)	(7.04 – 12.44)
Male (%)	53 (52.5)	12 (42.9)	41 (56.2)
Ethnicity			
White British (%)	98 (97)	28 (100)	70 (95.9)
Other (%)	3 (3)	0 (0.0)	3 (4.1)
Maternal education			
None (%)	2 (2.0)	0 (0.0)	2 (2.7)
GCSE /A-level or equivalent (%)	62 (62.0)	20 (74.0)	42 (57.5)
Graduate / Postgraduate (%)	36 (36.0)	7 (25.9)	29 (39.8)
Paternal education			
None (%)	8 (8.1)	3 (11.1)	5 (6.9)
GCSE /A-level or equivalent (%)	56 (56.6)	17 (62.9)	39 (54.1)
Graduate / Postgraduate (%)	35 (35.3)	7 (25.9)	28 (38.9)
Family history of food allergy			
Maternal (%)*	23 (22.5)	10 (35.7)*	13 (17.8)*
Paternal (%)	16 (15.6)	7 (25.9)	9 (12.3)
Sibling (%)*	18 (17.6)	10 (35.7)*	8 (11.0)*

GCSE = General Certificate of Secondary Education

Infant Feeding

Details of participants' infant feeding history are shown in Table 2. Significantly more of the control group had ever been breastfed compared to the CME group ($p = 0.02$) and they were breastfed for longer ($p = 0.017$). A greater proportion of those in the CME group had been fed with predominantly readymade baby food ($p = 0.018$).

Table 2: Infant feeding characteristics of participants. *significant difference between groups ($p < 0.05$)

	All (n = 101)	CME group (n = 28)	Control group (n = 73)
Ever breastfed			
Yes (%)*	78 (78.0)	17 (60.7)*	61 (84.7)*
No (%)	22 (22.0)	11 (39.3)	11 (15.3)
Ever given formula milk			
Yes (%)	91 (91.0)	28 (100.0)	63 (87.5)
No (%)	9 (9.0)	0 (0.0)	9 (12.5)
*Breastfeeding duration**			
Never (%)	22 (22.0)	11 (39.4)*	11 (15.3)*
< 6 months	45 (45.0)	14 (49.9)*	31(43.0)*
> 6 months (%)	33 (33.0)	3 (10.7)*	30 (41.7)
Age at introduction of solid foods (weeks)	16 (10-26)	16 (11-24)	16 (10-26)
Predominant type of weaning food			
Homemade (%)	50 (50.5)	15 (53.6)	35 (49.3)
Readymade baby food (%)*	5 (5.1)	4 (14.3)*	1 (1.4)*
A mixture of both (%)	44 (44.4)	9 (32.1)	35 (49.3)

Symptoms, dietary exclusion and skin prick test (SPT) status

The CME group reported significantly more symptoms than the control group ($p < 0.001$). The most commonly reported symptom overall was vomiting, reported by 59.4% of all participants (89.3% of the CME group and 47.9% of the control group). All participants in the control group who were formula fed were fed standard infant formula. Within the CME group the most commonly used formula was soya, used by 50% of the CME participants. In the CME group, substitute formula was initiated at a median age of 11.5 weeks (range 2-40), with a median duration of usage of 67.5 weeks (range 16-205 weeks). Four participants (14.3% of CME group) had a positive SPT to cows' milk. For those with a negative SPT, diagnosis of cows' milk allergy was made on the basis of clinical history and supervised dietary exclusion/reintroduction.

Food allergens excluded by the CME group are displayed in Figure 2.

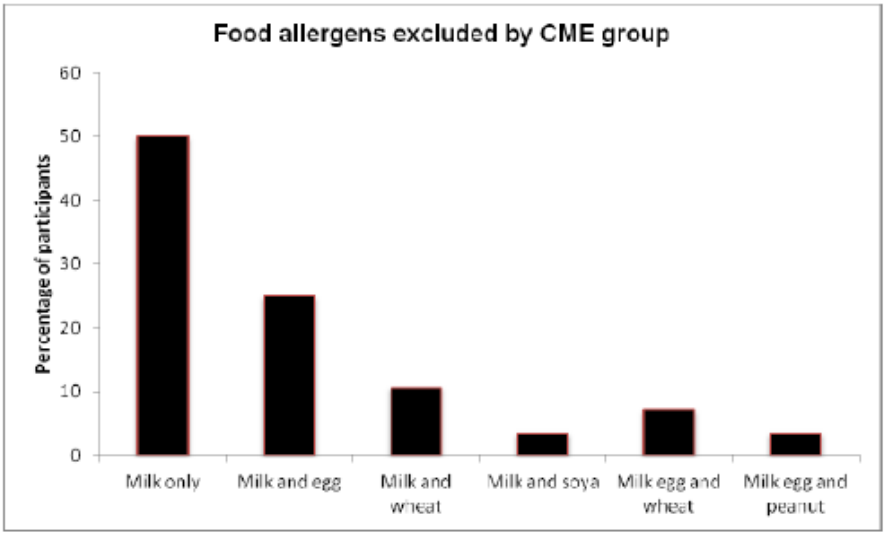

Figure 2. Type of food allergens excluded by CME group

Fussy Eating

There were no significant differences found for individual subscales of the CEBQ by gender or family history of food allergy and no association between questionnaire scores and participant age, parental education or occupation status or any infant feeding factors. Differences between the CME and control group for the fussy eating questionnaire are shown in Figure 3. The CME group had significantly higher scores for the "slowness in eating" subscale ($p < 0.001$). No significant difference was found for the other three subscales individually (food responsiveness, fussiness and enjoyment of food), however when the three negative subscales were combined to give a measure of "avoidant food behaviour", there were significantly higher scores observed in the CME group ($p < 0.001$). There was no significant difference in CEBQ scores between participants in the CME group according to type of formula consumed.

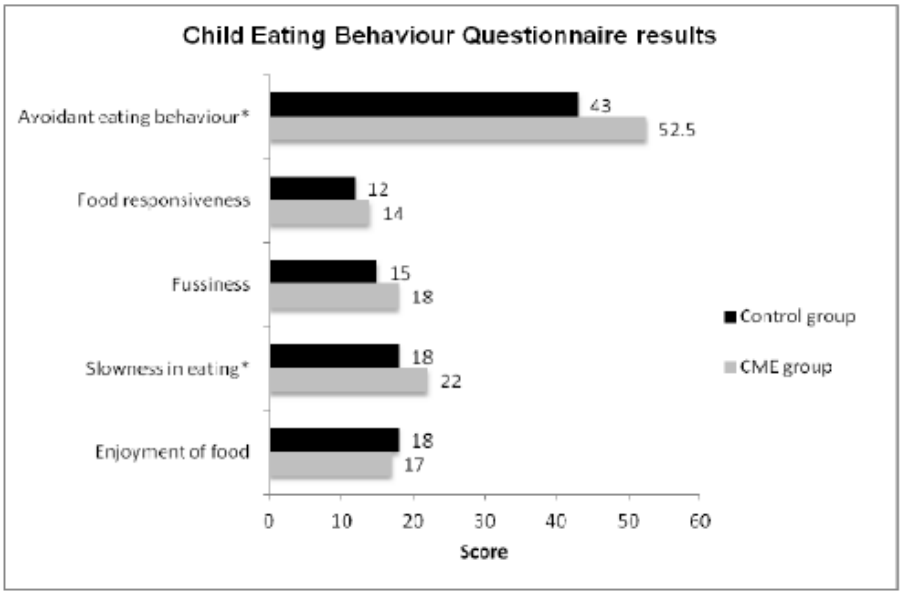

Figure 3. Results of the Child Eating Behaviour Questionnaire. *significant difference between CME and control group $p < 0.05$

The number of reported symptoms was moderately correlated with higher levels of avoidant eating behaviour, across all participants (rho = 0.272, p = 0.006). The number of food allergens excluded was moderately positively correlated with slowness in eating (rho = 0.283, p = 0.04) and avoidant eating behaviour (rho = 0.345, p = 0.000).

As breastfeeding duration and number of symptoms were found to be significantly different between the CME and control groups, two separate one-way ANCOVA calculations were conducted to compare the avoidant eating behaviour scores between dietary exclusion groups, whilst controlling for these two factors. After adjusting for breastfeeding duration, a significant difference between the CME and control groups persisted (p = 0.001), with dietary exclusion status explaining 11.2% of the variance in avoidant eating behaviour (p = 0.263, partial eta squared = 0.013). Likewise, after adjusting for the number of symptoms, a significant difference between the CME and control groups persisted (p = 0.005), with dietary exclusion status explaining 7.8% of the variance in avoidant eating behaviour (p = 0.230, partial eta squared = 0.015).

Food preference

Total liking for all foods was not found to be associated with age, number of symptoms or any infant feeding variables. Liking for all foods was inversely related to food fussiness (rho = -0.473, p = 0.0001) and slowness in eating (rho = -0.340, p = 0.001), but positively correlated to enjoyment of food (rho = 0.314, p = 0.002). The most preferred category by all participants was the sweet and fatty foods category and the least preferred category was vegetarian substitutes, followed by vegetables.

No difference was found between the CME and the control group for any category of food. However, looking at individual milk containing foods, significant differences were found between the CME and control groups for a number of foods, with the control group rating them more positively. This is shown in table 3. As was the case with fussy eating, there was no significant difference in food preference scores between participants in the CME group according to type of formula consumed.

Table 3. Difference between CME and control group preference scores for dairy foods.
*Rated more positively by control group, significance of $p < 0.05$

Food	p value
Full fat milk*	0.004*
Semi skimmed milk	0.164
Yoghurt	0.168
Butter*	0.043*
Margarine	0.243
Cream*	0.016*
Cheese	0.853
Ice cream*	0.030*
Chocolate*	0.024*

Discussion

This study set out to compare the eating habits of two groups of children: one group who had consumed a CME diet for CMA as infants and one group who had consumed an unrestricted diet as infants, but who are all now consuming unrestricted diets. As the majority of participants (96/101) were recruited from two large birth cohort studies it was possible to measure the effect of infant feeding variables on current eating behaviours using prospectively collected data from infancy. In terms of fussy eating, the CME group scored

significantly higher on "slowness of eating" and on the combined "avoidant eating behaviour" construct. There was no difference according to gender or infant feeding, but a higher number of excluded food allergens and symptoms were associated with more negative eating behaviour. The CME group rated liking of several dairy foods (butter, cream, chocolate, full fat milk and ice cream) significantly lower than the control group, although there were no significant differences seen for the overall dairy category or for any other category of food.

The significant difference observed between dietary exclusion groups for avoidant eating behaviour is a novel finding. It demonstrates a long-term effect of modifying the infant diet, persisting approximately 7-10 years, even whilst controlling for breastfeeding duration and number of allergic symptoms. The moderate correlations observed between both number of symptoms and number of avoided food and worse eating behaviour are also novel findings. The finding that those in the CME group are slower eaters is of note. Mealtime duration is associated with problem eating behaviour, with prolonged mealtimes known to worry parents (16). Although this study measured behaviour based on parent report rather than child report or direct observation of eating, the questionnaire used has been validated against behavioural measures of food intake and demonstrated to have good internal consistency, reliability and construct validity (17). In a recent study of eating behaviour, Adamson *et al.* found parents to be accurate reporters of mealtime duration and reported that children who were "problem eaters" spent more time engaged in aversive behaviour and less time eating.

Food preference is due to a combination of several properties (e.g. taste, olfaction, texture and temperature). The categorisation of foods into nine broad categories is arbitrary and is related to food type (e.g. meat, vegetables) not necessarily to taste or sensory properties. Because of the limitations with the grouping of individual foods into categories, a sub analysis was carried out on individual milk containing foods. Although there was found to be no difference in preference ratings between the groups for some milk containing foods (e.g. semi skimmed milk, yoghurt) significant differences were found for five milk containing foods (full fat milk, butter, cream, ice cream and chocolate). This is particularly surprising, considering that in Cooke *et al.*'s study of children consuming unrestricted diets (15) chocolate, pizza and ice cream were the three most favoured foods. Indeed, recent national UK dietary data reports that amongst young people aged 4-18 years, mean weekly intakes of chocolate and ice cream are 63-84g (~two standard bars) and 56-91g (~1.5 scoops) respectively (18). A high percentage of children's food preferences are formed by the age of 2-3 years (19) therefore if exposure and liking of these dairy products was not established in infancy due to CMA, it is understandable that they are now disliked. We hypothesise that

cheese, yoghurt and semi skimmed milk were introduced to the diet of children with resolving CMA as these were deemed to be nutritious foods, yet butter, cream, ice cream, full fat milk and chocolate were introduced at a later stage as they were perceived to be less healthy or less important.

Milk aversion is well documented in adults with suspected lactose intolerance and it is likely a learned trait caused by experiencing unpleasant side effects (20). The literature regarding children outgrowing food allergy has not specifically studied the role of food dislike or aversion. Kim *et al.*'s (12) study of children who had undergone baked milk challenges, observed that 12% of those who had tolerated baked milk products chose to avoid the food when reviewed five years later. Mostly this was attributed to a fear of recurrence of symptoms and all declined to have a further supervised challenge in hospital. A study of 210 children with CMA in Finland found that although 120 participants had introduced milk products by the age of three years, with 87% drinking milk, 67% consuming cheese and 45% consuming yoghurt; the total amounts of milk products consumed daily were small and less than the national average (21).

Limitations

There are some limitations to this study. Firstly the CME group was a heterogenous group as the two birth cohorts took place five years apart, involving two separate research protocols. However both cohorts took place in the same region and by incorporating both studies, allowed a broader age range and larger sample to be recruited. The most commonly used formula (soya), is no longer recommended as first line treatment of CMA (22). The lack of ethnic diversity and high maternal educational level means the study may not be generalisable to other populations, however previous international studies have shown that food preferences and fussy eating in children are consistent across cultures. Despite the power calculation indicating 129 participants were required, it was only possible to recruit 101. Although we did detect some differences between groups, the lack of power could explain why others were not observed. For example we did not observe any differences between formula types (soya compared to extensively hydrolysed) within the CME group. Maternal food preferences and food availability were not measured. Although we have reported differences in eating behaviour, we have not reported nutritional intake.

Conclusion

Overall this study demonstrated that consuming a CME diet during infancy has an effect on some, but not all, eating habits that were under investigation. However, given that the avoidance of cows' milk in the short term is currently the only management option for CMA,

this will not fundamentally change its management in routine clinical practice, although it does underline the importance of a robust diagnosis. The implications of these findings are that problem eating habits in children consuming CME diets need to be addressed early and appropriate dietetic intervention occurs during both the exclusion and reintroduction period.

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Appendix 2

Pediatric Allergy and Immunology

ORIGINAL ARTICLE Food allergy

Fussy eating and feeding difficulties in infants and toddlers consuming a cows' milk exclusion diet

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Keywords
cows' milk allergy; feeding difficulties; fussy eating; infant feeding

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Abstract

Background: Cows' milk allergy (CMA) is the most common infant food allergy in the United Kingdom, requiring a strict exclusion diet. Feeding difficulties and fussy eating are also very common problems in young children and can negatively influence feeding and dietary intake in an infant with CMA. The aim of this study was to compare the levels of fussy eating and feeding difficulties in two groups of young children: a group consuming an exclusion diet for CMA and a control group of children consuming an unrestricted diet.

Method: Participants were recruited from allergy and health visitor clinics on the Isle of Wight. Parents completed a number of questionnaires about their child's feeding behaviour.

Results: One hundred and twenty-six participants (mean age 13 months) were recruited. Participants consuming an exclusion diet for CMA had significantly higher scores for both fussy eating and feeding difficulties ($p < 0.05$), although overall both groups were within the normal range. A number of symptoms were found to be positively moderately correlated with higher feeding difficulty score ($p < 0.05$). A higher consumption of milk/milk substitute consumed per day was positively correlated to both feeding difficulties and fussy eating ($p < 0.05$).

Conclusion: Participants consuming an exclusion diet for CMA have higher scores for feeding difficulties and fussy eating than those consuming an unrestricted diet; however, the majority of participants' scores were within the normal range and did not affect the growth.

Cows' milk allergy (CMA) is known to affect ~3% of children in the United Kingdom (UK) (1). It is also known that parents may incorrectly perceive their child to have a food allergy (2) and that allergen avoidance diets are sometimes initiated unnecessarily (3, 4). In practice, this means that many children are excluding a major food group from their diet at a time in life that is critical for growth, development and establishment of eating habits. Infants with CMA who are not breastfed are prescribed hypo-allergenic infant formulae, which have an altered taste. Parents are also advised that their child should follow a special weaning diet avoiding all forms of cows' milk, usually until at least 1 year of age, but this exclusion diet may continue for much longer.

Fussy eating and feeding difficulties are separate entities that may co exist. Fussy eating, generally defined as 'consuming a limited variety of food' is a very common problem in young children (5). Up to 20% of infants and toddlers in the UK are reported to be 'problem' eaters by their parents (6) with some studies reporting up to 50% are fussy eaters (7). In healthy infants and toddlers, it is known that development of feeding skills occurs from 0 to 24 months with individual variation in gaining self-feeding fine motor skills (8). Feeding difficulties refer to a spectrum of problematic eating behaviours such as excessive spitting out of food, crying/irritability at feeding time, eating extremely slowly, retching at the sight of bottle or spoon, apparent difficulty in swallowing, throwing and pushing away food (9, 10). Feeding difficulties are known to be more common in certain medical conditions (e.g. autism spectrum disorder) (11).

In a young child with suspected or confirmed food allergy, where at least one food group is already being restricted, fussy eating and feeding difficulties are likely to have a considerable

impact on eating habits and food intake. To date, there has been limited research directly investigating the prevalence of these eating problems in children consuming a special diet for food allergy (12). The existing studies have mainly recruited children with severe non-IgE-mediated gastrointestinal disease and have not included a control group of children eating a normal diet (13, 14). The aim of this study was to determine the prevalence of fussy eating and feeding difficulties in infants and toddlers consuming a cows' milk exclusion (CME) diet compared to a control group consuming an unrestricted diet. If they are found to be more prevalent, intervention by a qualified dietitian will ensure timely diagnoses and appropriate advice to prevent long-term consequences of fussy eating habits.

Methods

Study design

This was a cross sectional study of 8- to 30-month-old children from the Isle of Wight, UK. This study included two groups: an experimental group, composed of children consuming a CME diet for the treatment of presumed CMA and a control group of children consuming an unrestricted diet. Children were eligible for inclusion in the experimental group if they had consumed a hypo-allergenic formula and/or a CME diet in the first year of life for a period of 3 months or longer and or if they were excluding other foods (e.g. egg or soya).

Recruitment took place between July 2013 and December 2014. Participants eligible for the experimental group were identified via routine allergy clinics. The control group was recruited from health visitor clinics in the same locality. Ethical approval was obtained from Berkshire NHS Ethics Committee.

Data collection

Fussy eating and feeding difficulties were measured using two separate questionnaires. Fussy eating was measured using the picky eater questionnaire (15). It consists of 10 items describing specific behaviours related to fussy eating with questions such as 'overall to what extent does your child like a wide variety of foods from those that you think he/she should eat?' and 'how often do you prepare a special food for your child because he/she does not like what the rest of the family is eating?'. Feeding difficulties was measured using the Montreal Children's Hospital feeding difficulty questionnaire (16). It consists of 14 comprehensive questions, covering the following feeding domains: oral motor, oral sensory, appetite, maternal concerns about feeding, mealtime behaviours, maternal strategies used and family reactions to child's feeding. Information was also collected on social demographics, family history of allergy, allergic symptoms, infant feeding and growth.

Data analysis

A power calculation for a two-tailed outcome at 80% power indicated that 124 participants were required in this study. Questionnaires were scored and coded according to published guidelines. Data were analysed using SPSS software (IBM, version 20 Armonk, NY, USA). Descriptive statistics were calculated. Differences between the CME and control groups were compared using Mann–Whitney *U*-test or χ^2 test. Spearmann rho correlations were performed. Multiple regression calculations were performed to determine the contributing factors to the main outcome variables. A significance level of $p < 0.05$ was set for all analyses.

Results

Description of sample

One hundred and twenty-six participants were recruited. Demographic characteristics are detailed in Table 1. Participants in the CME group were younger than those in the control group ($p = 0.02$), but the age range was the same. There were no differences in gender, number of siblings, ethnicity, maternal age/education or growth measurements between the two groups.

Infant feeding and dietary exclusion

Details of participants' infant feeding history are shown in Table 2. The majority of infants had been breastfed at some stage (81%), but only 13.5% were being breastfed at the time of data collection. Infants in the control group were commenced on solid food ($p = 0.033$), lumpy food ($p = 0.049$) and finger foods ($p = 0.000$) significantly earlier than the CME group.

71.2% of the CME group was excluding cows' milk only, whilst 28.8% were excluding another food allergen in addition to cows' milk. Cows' milk was excluded at a median age of 9.5 weeks (range 1–30). Three infants in the CME group were breastfed as their main source of milk and did not have any substitute formula. At the time of data collection, the median duration of a hypo-allergenic formula use was 41.0 weeks (range 2–91 weeks). The most commonly used hypo-allergenic formula was amino acid formula (45.5%), followed by extensively hydrolysed (EH) whey formula (25.8%) and EH casein formula (16.6%).

Reported symptoms and skin prick test status

Participants in the CME group reported a median number of 4.0 symptoms (ranging from 1 to 7 symptoms). Participants whose mother had a history of food allergy had significantly more symptoms reported ($p = 0.000$), with reported higher rates of vomiting ($p = 0.037$), abdominal pain ($p = 0.000$) and colic ($p = 0.004$) than those with no maternal history of food allergy. Twenty participants (30.3%) in the CME group had a positive skin prick test (SPT) to cows' milk (>3 mm). Participants who had a positive SPT to cows' milk reported significantly more symptoms ($p = 0.006$).

Main outcome measures

Feeding difficulties

The median feeding difficulty score in the CME group (26.5, range 16–68) was significantly higher than that of the control

Table 1 Demographic and anthropometric characteristics of participants

	All (n = 126)	CME group (n = 66)	Control group (n = 60)
Age in months (range)	13.0 (8–27)	12.37* (8–25)	15.0* (8–27)
Gender (n, %)			
Female	59 (46.8)	32 (48.5)	27 (45.0)
Male	67 (53.2)	34 (51.5)	33 (55.0)
Maternal age (years) mean	29.28 (s.d. 6.5)	29.8 (s.d. 6.38)	28.6 (s.d. 6.62)
Ethnicity (n, %)			
White British	118 (93.6)	61 (92.5)	57 (95.0)
Non-White British	8 (6.4)	5 (7.5)	3 (5.0)
Maternal education (n, %)			
None	1 (0.8)	0 (0.0)	1 (1.7)
GCSE/A-level or equivalent	80 (63.4)	41 (62.1)	39 (65.0)
Graduate/Postgraduate	41 (32.6)	23 (34.8)	18 (30.0)
Not stated	4 (3.2)	2 (3.0)	2 (2.3)
Birthweight (kg)	3.43 (1.55–4.67)	3.48 (2.08–4.67)	3.34 (1.55–4.53)
Weight (kg)	9.9 (7.43–14.90)	9.9 (7.59–14.9)	10.1 (7.43–14.9)
Length/height (cm)	76.0 (68–90.4)	76.0 (69.0 –90.4)	76.0 (68.0–88.0)
Weight/age z score (s.d. units)	0.31 (s.d. 1.01)	0.41 (s.d. 1.09)	0.18 (s.d. 0.89)
Height/age z score (s.d. units)	0.44 (−1.76–3.91)	0.46 (−1.76–3.91)	0.5 (−1.19–3.41)
BMI (kg/m$^{2)}$	17.0 (14–20.6)	17.1 (14–20.6)	17.0 (14.3–19.0)
BMI Z score (s.d. units)	0.39 (−1.95–2.58)	0.45 (−1.95–2.58)	0.12 (−1.39–1.9)

*Difference between CME group and control group significant <0.05.

Table 2 Infant feeding overall sample and by group

	All (n = 126)	CME group (n = 66)	Control group (n = 60)
Currently being breastfed (n, %)			
Yes	17 (13.5)	8 (12.1)	10 (16.7)
No	108 (85.7)	58 (87.9)	50 (82.3)
Ever been breastfed (n, %)			
Yes	102 (81.0)	54 (81.8)	48 (80.0)
No	24 (1.09)	12 (18.2)	12 (20.0)
Ever given formula milk (n, %)			
Yes	115 (91.3)	64 (97)*	50 (82.3)*
No	11 (8.7)	2 (3.0)	10 (16.7)
Age at introduction of solid food (weeks)	20 (5.5–30)**	20.0 (5.5–30)**	18 (12–26)**
Age at introduction of lumpy foods (weeks)	26 (12–42)**	26.5 (12–42)**	25 (16–36)**
Age at introduction of finger food (weeks)	26 (16–52)***	26.5 (20–52)***	24 (16–36)***
Type of weaning food (n, %)			
Homemade	64 (50.8)	33 (50.0)	31 (51.7)
Prepared baby food	9 (7.1)	7 (10.6)	2 (3.3)
A mixture of both	53 (42.1)	26 (39.4)	27 (45.0)

*Difference between CME group and control group significant <0.01 using a chi-squared test.
**Difference between CME and control group significant <0.05 using a Mann–Whitney *U*-test.
***Difference between CME and control group significant <0.01 using a Mann–Whitney *U*-test.

group (22.0, range 15–53) ($p < 0.01$), although both groups were within the normal range (<45). Nine participants in the CME group (13.6%) had scores diagnostic of clinical feeding difficulties (>45), compared to only one participant in the control group (1.6%). There was no affect of gender, being older or younger than 12 months, or breastfeeding status on feeding difficulty score. Participants whose mothers had a history of food allergy symptoms recorded significantly higher scores of feeding difficulties ($p = 0.03$).

Within the CME group, there was no correlation between feeding difficulty score and age at introduction of hypo-allergenic formula, duration or type of hypo-allergenic formula consumption or SPT status. However, some symptoms were found to be significantly correlated with a higher feeding difficulty score. These are listed in Table 3. In addition, the amount of milk substitute formula consumed per day and 'attention paid to healthy eating' were also found to be significantly correlated to a higher feeding difficulty score as

was a younger age at time of initiating the exclusion diet. Maternal age, growth, parental education, the number of siblings, duration of breastfeeding, age of introduction of solid/lumpy food and duration of exclusion diet were not correlated with feeding difficulty score.

A standard entry multiple regression analysis was undertaken on the CME group to determine the ability of several factors to predict the level of feeding difficulties. In the final model, 41.3% of the variance in feeding difficulties could be explained (R = 0.642, s.e. 11.09). A history of colic made the most contribution to this model (B score = −0.459, p = 0.03). Three variables made a unique statistically significant contribution (colic, dry cough at night and other food-related problems). Details are shown in Table 4.

Fussy Eating

The CME group had a significantly higher median score (22.5, range 10–63) than the control group (18.0, range 10–44) ($p < 0.01$), indicating they have higher levels of fussy eating, although both groups' median scores could be considered in the non-fussy range (15). Overall, there was no difference in scores for gender, being older or younger than 12 months, maternal food allergy history or breastfeeding status. Within the CME group, there was no correlation between fussy eating score and age at introduction of hypo-allergenic formula, duration of hypo-allergenic formula consumption, type of hypo-allergenic formula, growth or SPT status. A positive correlation existed for volume of milk substitute consumed per day (Table 3).

Discussion

This study set out to compare level of feeding difficulties and fussy eating in two groups of young children; one group consuming a CME diet for CMA and a control group consuming an unrestricted diet. Overall, we demonstrated that the CME group scored significantly higher for fussy eating and feeding difficulties, although the results for both groups were within normal ranges. Feeding difficulties were found to be significantly positively correlated with a number of allergic symptoms, and both variables were found to be correlated with a higher volume of milk substitute consumed per day.

Table 3 Factors correlated to a higher feeding difficulty or fussy eating score (Spearmann rho)

	Feeding difficulty	Fussy eating
Wheezing/whistling in chest	0.357**	0.239
Dry cough at night	0.358**	0.231
Itchy rash coming and going	0.228	0.212
Vomiting	0.198	0.109
Diarrhoea	0.380	0.077
Constipation	0.189	0.139
Abdominal distension	0.060	0.084
Colic	0.252*	0.172
Other food-related problems	0.145	0.002
Number of symptoms	0.355**	0.218
Volume of milk substitute per day	0.327**	0.305*
Attention paid to healthy eating	0.251*	0.147
Age at time of dietary exclusion	−0.249*	−0.105

**$p < 0.01$; *$p < 0.05$.

Table 4 Multiple regression model explaining 41.3% of variance in feeding difficulty scores in cows' milk exclusion group

	B	s.e. β	β	*t*	p
Number of symptoms	−2.814	1.840	−0.351	−1.529	0.132
At what age was the child first given lumpy food (weeks)	0.460	0.254	0.195	1.810	0.076
Wheezing or whistling in the chest	−9.035	4.504	−0.285	−2.006	0.050
Dry cough at night,	−14.991	4.539	−0.448	−3.303	0.002
Maternal food allergy	3.249	3.186	0.124	1.020	0.312
Vomiting	−5.675	3.823	−0.199	−1.484	0.144
Constipation	−3.349	3.508	−0.125	−0.955	0.344
Colic	−11.390	3.622	−0.459	−3.145	0.003
Other food-related problems	−7.630	3.133	−0.286	−2.435	0.018

The higher scores observed on the feeding difficulty questionnaire in the CME group was statistically significant. This is the first time this has been reported in a study of infants with suspected CMA using a control group and a validated questionnaire. However, it should not be overlooked that both groups had median scores well within normal levels. Indeed, the number of children in the control group with feeding difficulties (1.6%) is considerably lower than that reported in previous studies of normal healthy developing children (6, 9); however, the methodology for those studies was different.

Studies of feeding difficulties and food allergy have typically been conducted on children with complex gastrointestinal allergies (13, 14, 17), or in children who also have an underlying comorbidity (18), therefore the participants are not necessarily reflective of the 'typical' infant with CMA. Meyer et al. (n = 437) found that 30–40% of children with food protein-induced gastrointestinal allergies (FPIGA) had feeding difficulties reported in their medical notes, with a higher rate in those with symptoms of abdominal pain, vomiting, bloating and constipation. Although there are differences between that study and this; there are some commonalities. They identified a significant correlation between feeding difficulties and extra-intestinal manifestations (joint pain, lethargy and headaches). Likewise, this study identified a significant correlation between non-gastrointestinal allergic symptoms (wheeze and cough) and feeding difficulty score, illustrating that childhood eating/feeding habits are influenced by a wide range of health-related factors. It is known that oral eating requires the coordination of a suck-swallow-breathe pattern, and it may be that difficulties in sensory processing are related to cardiorespiratory symptoms including those present in asthma (19). Feeding difficulties are also reported in children with other respiratory conditions (20, 21).

Similar to the study by Crist et al. (9), feeding difficulty score was not found to be related to socio-economic status or

birth order/number of siblings. Contrary to previous studies (22, 23), a link between the age of introduction of any type of solid foods and feeding difficulty score was not identified. Introduction of lumpy foods did contribute to the multiple regression model predicting higher feeding difficulty score, however, only in combination with other variables. However, it must be highlighted that the reporting of age of introduction of solid food was based on parent recall, which may affect the accuracy of this data.

Overall, infants in the CME group scored significantly higher on their fussy eating questionnaire than the control group. However, the median score of 22.5 is still well below the maximum questionnaire score of 70, indicating that, as a whole, the group was not particularly fussy eaters. In a previous study of 2- to 3-year-old children, 'picky eaters' were found to have a mean score of 34.3, compared to 'non-picky eaters' who had a mean score of 22.7 (15). A study of 12-month-old infants examining the role of food texture and fussiness reported a mean score of 25 on a subscale of the questionnaire (24), which is similar to our findings.

No correlations were identified between fussy eating and allergic symptoms. A recent study of 4-year-old children in Holland identified a bidirectional correlation between constipation and fussy eating (25). They found no difference in fussy eating levels between those with and without CMA history (personal communication Tharner, January 2015). Other studies have reported that fussy eating occurs across different socio-economic statuses, genders, ethnic groups and ages (15), which is consistent with our findings. Across all participants, no difference in fussy eating score was found in relation to maternal age or education/occupation status. It is notable that the total volume of milk/milk substitute consumed/day was positively correlated with fussy eating score. This supports the simple dietetic advice to reduce excessive consumption of formula to encourage a better appetite and mealtime behaviour.

Fussy eating can be difficult to quantify accurately and is usually evaluated by a parental report tool or asking of a single yes/no question, rather than analysis of dietary records (26). Although several tools have been developed for measurement of preschool children's fussy eating behaviour, none have been specifically designed for children under 18 months old and this was identified as a gap in the literature in a recent review (27). The questionnaire used in this study was chosen as it has been validated against behavioural measures of eating in 12-month-old infants (24) and against two types of dietary records in children aged 24–36 months old.

The measurement of feeding difficulties can also be problematic due to the variability in definitions used. In many cases, feeding difficulties are transient; however, it is not always straightforward to distinguish feeding problems that are likely to be short-lived from those that are more persistent (28). By comparison, the term 'infant feeding disorder' is a formal diagnosis used in the current diagnostic systems of the World Health Organization ICD-10 (29) and Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (30). Both sets of criteria specify that an infant feeding disorder is a persistent failure to eat adequately, associated with weight loss/significant failure to gain weight, that is not directly due to a medical condition or another mental disorder, with onset before 6 years of age. As many children who consume exclusion diets maintain a normal weight and have an underlying disorder (i.e. food allergy), the use of this definition was not appropriate for this study. Other classification systems such as the Chatoor criteria and Wolfson criteria (31) have been developed, but both involve lengthy questionnaires. The Montreal Hospital Children's feeding scale questionnaire is, to the authors' knowledge, the only validated questionnaire for measurement of feeding difficulties in children under 2 years of age (16). It is an easy-to-use measurement that has been demonstrated to be valid and reliable in children with and without medical diagnoses and could be quickly administered in an outpatient setting, in approximately 5 min, with good reliability and internal consistency.

Limitations and strengths of study

There are some limitations to this study. There may be a recruitment bias whereby those more interested in diet are more likely to participate. The method used is reliant on subjective parental report. Parental feeding behaviours, which have the potential to influence infant feeding behaviours (32) were not assessed. The control group was slightly older than CME group, which may have skewed the results slightly. The CME group included participants consuming both single and multiple exclusion diets. As this was a typical caseload of patients from a secondary care allergy clinic, participants were diagnosed with CMA using clinical history, SPT and dietary exclusion/reintroduction, rather than an oral food challenge. As correlations are reported, causality cannot be confirmed.

The strengths of this study are the use of a control group, which was recruited from the same geographical locality as the CME group. The groups were closely matched for all demographic variables; only participant age differed by 3 months. As the research took place in a secondary care allergy clinic, the results are broadly generalizable to the majority of other clinics around the UK. The fact that the infant feeding data of the group as a whole are so similar to national feeding trends demonstrates that the control group is also reflective of the general population. The recruitment target of the study was met, meaning the study was sufficiently powered. Validated and age-specific questionnaires were used. Data collection, coding, analysis and interpretation took place by the same researcher to minimize the effect of researcher bias.

Conclusion

In summary, it has been demonstrated that infants consuming a CME diet for CMA have significantly higher scores of feeding difficulties and fussy eating than a control group consuming an unrestricted diet. This may be due to the underlying disease process resulting in allergic symptoms, the restrictive nature of the CME diet or due to feeding practices

adapted by the parent and child. The number of allergic symptoms was the factor that was most strongly correlated with feeding difficulties; however, type of symptoms was also important, as was the volume of milk substitute consumed per day. However, it should be emphasized that the feeding difficulties and fussy eating scores across the whole group were within normal ranges, and there was no effect seen on growth. This provides reassurance to health professionals who assess and advise parents of children with food allergy.

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Appendix 3

Infantile Colic – a guideline emphasising simple measures of support – and when Cows' Milk Allergy should be considered the cause
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Key learning points

1. Infantile Colic is common and often causes both infants and their carers considerable distress.
2. The cause or causes remain largely unexplained and there is little evidence to support therapeutic interventions. For most infants the key management approach is to advise simple measures and to ensure there is appropriate support in place for families.
3. In a small subset of infants, the cause will be Cows' Milk Allergy (CMA) and it is important to recognise which infants need this diagnosis to be properly explored.

Introduction

Colic is characterised by inconsolable excessive crying in the early weeks of life in an otherwise healthy infant. It is a common problem, estimated to effect between 5-19% of infants (1). Although the exact causative mechanism of colic remains unclear, it has been hypothesised that in some cases dietary allergens may be implicated in its cause and treatment (2). This article will provide an overview of colic and explore the possibility of a link between colic and Cows' Milk Allergy (CMA).

Diagnosis of colic

Although different definitions of colic exist, the most commonly accepted definition was first published in 1954 by Wessel et al. (3) and follows a 'rule of threes': "unexplained crying lasting > three hours a day, for > three days a week for > three weeks of duration". During the episodes of crying, infants may also draw up their legs, arch their back, have a flushed face, pass wind and have a rigid abdomen. Infants presenting with these symptoms should be assessed by a qualified medical practitioner to rule out other diagnoses (4). In some cases, excessive crying may mean an infant is presenting in pain, needing urgent investigation (see "warning signals" in Figure 1). Box 1 lists factors that should be considered before making a diagnosis of infantile colic.

History taking and examination in suspected infantile colic

- General health of the infant
- Antenatal and perinatal history
- Onset and length of crying
- Stool pattern (is the infant constipated?)
- Feeding assessment (check feeding technique)
- Maternal diet if breastfed (is it high in caffeine, carbonated drinks or spicy food?)
- Family history of allergy
- Parent's response to colic episodes
- Factors which lessen or worsen the crying episodes

Box 1. History taking and examination in suspected infantile colic (4)

Natural history and consequences of colic

Colic is thought to be largely a benign and transient condition that presents in the first 6 weeks of life and resolves spontaneously by approximately four months of age. Despite its short-lived nature, understandably the repeated bouts of crying can cause considerable parental distress. Perhaps unsurprisingly unexplained crying is the most common presentation to paediatricians in the first 16 weeks of life (5). In general, colic is not thought to have any long term health consequences, however it has been suggested that infants with colic may be more likely to have feeding difficulties (6) and that infants with severe cases of colic may be more likely to

experience recurrent abdominal pain and allergic disorders at the age of 10 years old (7).

Causes of colic

Despite several decades of research, the precise cause or causes of colic remain unknown. Numerous mechanisms have been proposed; including food hypersensitivity, gut dysmotility or immaturity, behavioural factors, maternal smoking and altered gut microflora, however it is possible that the cause is multifactorial, with inconsolable crying as the final common outcome (8). Breastfeeding does not appear to be protective against colic, as colic affects both breast and formula fed infants equally (1).

Colic as a presentation of Cows' Milk Allergy

In the UK, it is estimated that approximately 3% of infants experience CMA (9). Infants are exposed to cows' milk protein via the maternal diet if breastfed, via standard infant formula, or when solids are introduced. It is therefore not surprising that cows' milk is often identified as a possible cause for gut and skin problems, particularly in early infancy. It is known that parents may incorrectly perceive their child to have a food allergy (10) and that cows' milk free diets are sometimes initiated unnecessarily (11,12).

As can be seen in Table 1, infantile colic is listed by the National Institute of Clinical Excellence (NICE) food allergy guideline as one of the symptoms of food allergy (13). However, infants with CMA don't often present with colic as an isolated symptom. Typically, there may be some other skin and/or respiratory symptoms in addition to gastrointestinal symptoms.

Gastrointestinal	Skin	Respiratory
Gastro-oesophageal reflux disease Loose or frequent stools Blood and/or mucus in stools Abdominal pain ***Infantile colic*** Food refusal or aversion Constipation Perianal redness Angioedema of the lips, tongue and palate Oral pruritus Nausea Vomiting Diarrhoea Faltering growth plus one or more gastrointestinal symptoms above	Pruritus Erythema Acute urticaria Acute angioedema (most commonly in the lips and face, and around the eyes) Atopic eczema	Upper respiratory tract symptoms – nasal itching, sneezing, rhinorrhoea or congestion (with or without conjunctivitis) Lower respiratory tract symptoms (cough, chest tightness, wheezing or shortness of breath)

Table 1. Signs and symptoms of food allergy (13)

The NICE guideline (13) emphasises that food allergy should be particularly considered:
1) in infants where there is a family history of allergic disease (but no family history of allergy does not exclude the possibility of becoming allergic)
2) in infants where symptoms are persistent and affecting different organ systems and
3) in infants who have been treated for moderate to severe atopic eczema, Gastro Oeosphageal Reflux Disease (GORD) **or other persisting gastrointestinal symptoms (including 'colic'**, loose stools, constipation), but have not responded to the usual initial therapeutic interventions.

Colic and exclusion diets

Studies of exclusion diets, both maternal and infant, have yielded conflicting results, perhaps because many of the studies have small sample sizes and are prone to bias (14). In one study, maternal consumption of cruciferous vegetable (e.g. broccoli, cabbage, cauliflower) and onions was associated with increased colic, with no effect of chocolate or garlic (15). A systematic review concluded that changing the maternal diet to reduce the burden of allergy-associated foods can provide some benefit in reducing infantile colic in breasted infants (14). However, this must be weighed against the difficulties and practicalities of ensuring a balanced and adequate maternal diet to meet the demands of breastfeeding when excluding major food groups. In addition, it has been acknowledged that a placebo effect is often seen, with improvements in colic symptoms also reported in the control group of infants (16).

The evidence from the systematic review also suggests that the use of hydrolysed infant formula can be effective in reducing the symptoms of infant colic in formula fed infants, however consideration should be given to the resource and cost implications of such a measure. The NHS guidelines on routine postnatal care of women and their babies (4) which are due for review in September 2014, state that "use of hypoallergenic formula in bottle fed babies should be considered, but only under medical guidance". Unsupervised dietary exclusions can put infants at risk of nutritional deficiencies (17) or at risk of a more serious allergic reaction when cows milk is reintroduced (18), hence they should only be initiated under the advice and guidance of an experienced dietitian.

Case study one

Lucy was born at full term and was exclusively breastfed. From the age of four weeks she began to develop symptoms of colic, crying inconsolably for several hours every evening. Usual soothing techniques and colic drops were unsuccessful in reducing Lucy's crying episodes. Lucy continued to feed on demand and her weight gain was tracking along the 25th centile, but she regurgitated large volumes after each feed and her stools were very hard and difficult to pass. Lucy developed eczema at seven weeks of age. The areas of eczema on her face became more inflamed and irritated after each feed. Lucy was brought to her GP at 10 weeks old, who recommended a strict maternal milk free diet for four weeks, with a prescription of a calcium and vitamin D supplement. Although Lucy's mother found the exclusion diet difficult to follow initially, after four days there was a considerable reduction in the length of Lucy's crying. Her stools become softer and easier to pass, she was no longer regurgitating her feeds and the eczema on her face had cleared. Lucy was referred to a paediatrician and dietitian at age 14 weeks where the diagnosis of Cows' Milk Allergy was confirmed and appropriate weaning advice was provided.

Management and treatment of colic

NHS guidelines recommend that colic is best managed by providing parental reassurance that colic is a phase that will resolve spontaneously (4It emphasises the importance of peer support and suggests that such measures as gentle motion, 'white noise', baby massage and holding the infant may provide some comfort and relief during the crying episodes. There is insufficient evidence that medical treatments, such as lactase and simeticone drops are effective and they should only be tried if parents are unable to cope despite advice and reassurance, and discontinued if there is no improvement after one week. A systematic review of manipulative therapies (chiropractic, osteopathy and cranial manipulation), found some reduction in crying, however overall the studies had too few participants and were of insufficient quality to recommend manipulative therapies as a treatment for colic (8). The accompanying algorithm (Figure 1) provides a summary of the diagnosis and management of infantile colic.

Case study two

William was formula fed from birth and fed approximately every 3-4 hours. From three weeks old he became increasingly uncomfortable after each bottle, he passed large amounts of wind and began to refuse some feeds. Around six weeks old the symptoms worsened and he became very unhappy, crying for prolonged periods of time inexplicably between feeds. This caused a lot of stress for his parents, who suspected his milk was the cause of his symptoms. Three different brands of formula were trialled, including a 'comfort' milk, with no improvement. William was brought to his GP at nine weeks of age and was diagnosed with colic. There was no family history of atopy and William did not develop eczema at any stage. William's symptoms worsened over the next few weeks and he was brought back to his GP aged 13 weeks. He was given a two week trial of a hypoallergenic formula milk, which appeared to improve his crying bouts a little, but overall there was no dramatic improvement, indicating William did not have CMA. William returned to his usual formula milk aged 15 weeks. At 17 weeks, William's symptoms began to settle and he was sleeping better at night. At 21 weeks, William was started on a normal weaning diet.

Conclusion

In the majority of cases, colic is a transient and self-resolving condition that is not related to food allergy. However, in infants with persisting symptoms of colic, particularly if there are other symptoms suggestive of CMA, a 2-4 week trial of a maternal milk free diet or hypoallergenic formula is indicated. This should be supervised by a healthcare professional with knowledge of food allergy.

Unfortunately despite extensive research, the exact cause of colic still remains unknown in most cases. The most practical treatment advice at present is to provide parental reassurance, advise simple measures of management and to ensure that there is appropriate support for the family. Therefore we have developed a combined diagnostic and management algorithm for infantile colic that attempts to set out such a practical approach (Figure 1).

Useful Resources:

- UK National Support Group http://www.cry-sis.org.uk/
- For further information on managing infants with cows' milk allergy in primary care, please see: Venter et al. Diagnosis and management of non-IgE-mediated cow's milk allergy in infancy - a UK primary care practical guide. *Clinical and Translational Allergy* 2013; 3:23. Available online at: http://www.ctajournal.com/content/3/1/23

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Appendix 4

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Clinical and Translational Allergy

RESEARCH Open Access

Food allergy competencies of dietitians in the United Kingdom, Australia and United States of America

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Abstract

Background: A knowledgeable and competent dietitian is an integral part of the food allergy multidisciplinary team, contributing to effective diagnosis and management of food allergic disorders. Little is currently known about the food allergy training needs and preferences of dietitians. The purpose of this paper is to measure and compare self-reported food allergy competencies of dietitians based in the UK, Australia and USA.

Methods: A survey of USA-based paediatric dietitians was developed to measure self-reported proficiency and educational needs in the area of food allergy. The survey was modified slightly and circulated online to paediatric and adult dietitians in the UK and Australia. Descriptive statistics and Pearson correlations are presented.

Results: A total of 797 dietitians completed the questionnaire. Competency in "developing food challenge protocols" and "managing feeding problems" were rated the poorest overall across all three settings. A higher level of competency was significantly positively associated with length of practice as a dietitian, percentage of caseload composed of patients with food allergy and training in food allergy. The most popular topics for further training were food additives, pharmacological reactions and oral allergy syndrome.

Conclusions: There is a need amongst dietitians to increase their knowledge in different aspects of food allergy diagnosis and management, specifically the areas of developing food challenge protocols and management of feeding problems. This study provides valuable information for designing targeted food allergy education for dietitians.

Keywords: Competency, Dietitian, Food allergy, Knowledge

Introduction

The main aim in the management of Food Hypersensitivity (FHS) is to prevent the occurrence of acute and chronic symptoms by avoiding the offending food(s), whilst providing a nutritionally balanced diet [1]. In order to ensure effective management of any type of food allergic disorder, an appropriate dietary assessment and avoidance strategy is required [2]. A knowledgeable and competent food allergy dietitian is uniquely qualified to deliver this [3]. In recent years, five official international guidelines have been published on the diagnosis and management of food allergies; the World Allergy Organisation (WAO) guidelines on the diagnosis and management of cow's milk allergy [4], the USA National Institute of Allergy and Infectious Disease (NIAID) guidelines on the diagnosis and management of food allergies in adults and children [5], the UK National Institute of Health and Clinical Excellence (NICE) guidelines on the diagnosis of food allergies in children [6], the European Society for Paediatric Gastroenterology, Hepatology and Nutrition guidelines on Cow's Milk Protein Allergy [7] and the Irish Food Allergy Network (IFAN) Paediatric Food allergy guidelines [8]. Although each of these guidelines identifies the

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importance of a nutrition consultation, only the UK NICE, ESGPHAN and IFAN guidelines recognise that dietitians play a key role in both the diagnosis and management of food allergies.

In practice, the role of the dietitian working in the area of food allergy involves a range of responsibilities, consisting of, but not limited to [9]; taking an allergy-focused diet history and interpretation of skin prick tests, advising on formula choice and complementary feeding including nutrient supplements, allergen avoidance advice including practical advice on substitutes and recipes and monitoring nutritional status. Crucially, the dietitian has a lead role in the planning and design of food challenges for both diagnosis and determination of tolerance. A double blind placebo controlled food challenge remains the gold standard for diagnosis of food allergy [10]. Although in clinical practice, food challenges are typically not double blinded, expertise is required to calculate and translate appropriate doses to acceptable portion sizes. However, a previous survey of dietitians in the USA [11] indicated that despite good knowledge levels in some aspects of food allergy, a significant number of dietitians had no proficiency in developing food challenge protocols. This paper will compare self-reported food allergy competencies of dietitians based in the UK, Australia and USA, by combining previously published data from US –based paediatric dietitians [11] with new data which surveyed both adult and paediatric dietitians based in Australia and the UK.

Methods

The original survey of USA-based paediatric dietitians undertaken by Groetch *et al.* [11] was developed by a group of expert health professionals from the Consortium of Food Allergy Research (CoFAR), to measure self-reported proficiency and educational needs and preferences of paediatric dietitians. It was piloted, then distributed online to the Paediatric Nutrition Practice Group of the Academy of Nutrition & Dietetics. Respondents were asked to rate their knowledge and competency on a four point scale (high, moderate, low and not at all proficient). Permission to use this data as a published resource, in combination with newly collected data, was granted.

For both the UK and Australia, the questionnaire was modified to address local conditions. A five-point scale was used (high, moderate, low, not at all proficient and N/A in my practice). The questionnaire used in the UK is shown in Additional file 1: Table S1. The questionnaire used in Australia differed slightly as it had separate questions about Food Allergy (FA) and Food Intolerance (FI), where the term 'food allergy' was solely used for describing IgE mediated food allergy.

Sample

The distribution of the survey differed between countries. In the UK, a weblink was posted on the British Dietetic Association's (BDA) website, which has approximately 7000 members. The questionnaire was also published once in the BDA magazine and emailed once to dietitians who are members of specialist groups.

In Australia the questionnaire was circulated once via a weekly newsletter to all Dietetic Association of Australia (DAA) members, which has approximately 5000 members. A reminder email was sent three weeks later to the Food Allergy and Intolerance, Gastroenterology and Paediatric and Maternal Health Interest groups.

In the UK, the University of Portsmouth ethics committee was consulted, who advised that specific ethical permission was not required to undertake an online survey. In Australia, ethical approval was obtained from the Research Development Office of the Royal Prince Alfred Hospital, New South Wales.

Descriptive statistics are presented. Percentage responses are calculated per question based on the number of respondents answering the question. All statistical analyses were conducted using SPSS version 20.0 (SPSS, Inc., Chicago, ILL, 2012). One-tailed Pearson correlations were calculated to determine if any factors were associated with higher levels of competency.

Results

Participant characteristics

A total of 797 dietitians completed the questionnaire. Demographic characteristics of all participants are shown in Table 1.

A considerable number of participants worked in an outpatient setting (39%, 42% and 46% of UK, Australia and USA-based dietitians respectively). The majority of dietitians based in UK (58.3%) and Australia (77%) learnt about FA during their basic dietetic training. However in the USA, the majority of respondents (51.7%) learnt about allergy after qualifying as a dietitian.

The results of the UK and Australia questionnaires are compared with the results previously published by Groetch *et al.*, [11] in Table 2.

Food Allergy topics with high level of competency

Topics that were rated as "high" levels of competency are displayed in Figure 1. The USA-based dietitians had the greatest proportion of respondents rating themselves as highly competent for 6 areas (understanding definitions of FA and FI, recognising signs and symptoms, educating patients on avoidance, managing multiple food allergies and managing feeding problems). UK-based dietitians had the greatest proportion of respondents rating themselves highly in 2 areas (understanding diagnosis of

Table 1 Demographic characteristics of all participants

Characteristic	Options	UK (n = 336) %	Australia (n = 150) %	USA (n = 311) [11] %
Years in practice	0-5 years	31.7	42.0	20.6
	6-10 years	21.7	18.7	14.8
	11-15 years	15.5	12.0	15.1
	>15 years	31.8	27.3	49.5
Practice settings	Hospital (outpatient)	39.0	42.0	46.0
	Hospital (inpatient)*	NA*	40.0	37.6
	Private practice	2.7	32.0	13.2
	Community	36.0	34.0	-
	Industry	0.0	2.0	-
	Food Service	0.0	4.6	-
	Academic	0.3	2.6	-
	Research	0.9	5.3	-
	Other	21.1	4.6	28.3
Caseload composed of food allergy patients**	<10%	31.0**	66.0	57.6
	>10%	69.0**	34.0	42.4
Allergy training	During dietetic training	58.3	77.0	31.0
	Post registration course	17.0	28.0	51.9
	Postgraduate course	5.1	3.0	NA***
CPD resources currently used****	Academic journals	-	89.0	85.1
	Academic websites	-	52.7	59.3
	Dietetic/advocacy groups	-	70.1	72.0
	Conferences	-	70.0	56.0

NA = Not Applicable.
*UK questionnaire did not specify inpatient or outpatient.
**The UK respondents were not directly asked the proportion of their caseload comprised of FA patients. These figures relate to respondents who answered "not at all" or "slightly relevant" to the question "How relevant/applicable to your practice were the questions in this survey?".
***USA questionnaire did not list "postgraduate course" as an option.
****UK questionnaire did not ask what CPD resources currently used.

Table 2 Comparison of food allergy knowledge and competencies of dietitians based in the UK, Australia and USA

	High			Moderate			Low			Not at all		
	Aus	USA	UK	Aus	USA	UK	Aus	USA	UK	Aus	USA	UK
Understand FA	25	57	23	45	41	53	18	2	17	8	0	5
Understand FI	43	59	22	45	39	54	10	2	18	1	0	4
Understand diagnosis of FA/FI	18	19	23	41	53	41	30	24	26	8	4	6
Recognise signs and symptoms of FA/FI	25	29	23	50	58	48	20	12	22	3	2	4
Develop Food Challenge protocols	13	8	8	35	35	25	35	38	32	13	19	23
Educate patients on avoidance	30	42	33	39	46	42	25	12	18	3	1	4
Develop elimination diet	18	14	21	23	40	25	22	31	12	18	15	10
Manage Multiple FA	17	28	21	17	49	23	26	20	13	19	3	12
Manage Feeding problems	13	17	9	19	39	25	26	33	19	23	10	13

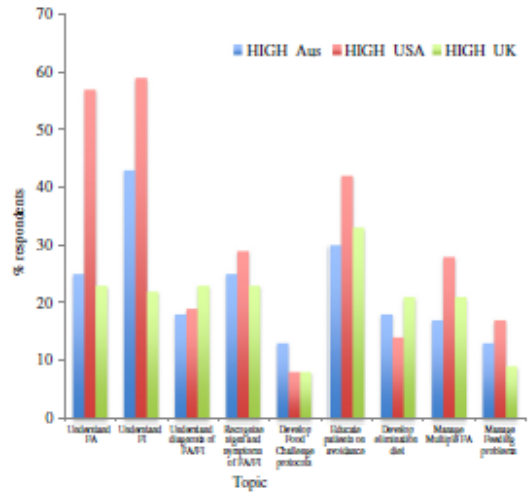

Figure 1 Food Allergy topics rated with "high" level of competency.

FA & FI and developing an elimination diet). Australia-based dietitians had the greatest proportion of respondents rating themselves highly for one area (developing food challenge protocols), however this was only 13% of respondents.

Food Allergy topics with low levels of competency

The competencies that were rated the poorest overall across all three countries were developing food challenge protocols and managing feeding problems, with 19% and 13% of all respondents respectively rating themselves as "not at all proficient".

However Pearson correlations calculated for the UK and Australia data indicate that higher competency in the areas of food challenge and managing feeding problems were significantly positively associated with length of practice as a dietitian, percentage of caseload composed of food allergy patients and training in food allergy. The strongest correlation existed between higher competency in managing feeding problems and% of caseload composed of allergy patients ($r = 0.50$, $p < 0.01$ in UK and $r = 0.517$, $p < 0.01$ in Australia). There was no correlation between competency in these two areas and setting of workplace. Correlation coefficients are displayed in Table 3.

Further training needed

Respondents in the UK and Australia were asked which specific FA topics they would like further training in. Results are shown in Table 4. Of note, the most popular topics were: reactions to food additives (67% and 73% in the UK and Australia respectively), pharmacological reactions (66% and 70% in the UK and Australia respectively) and oral allergy syndrome (62% and 68% in the UK and Australia respectively).

Educational resources needed

When asked what resources they would be "very likely" or "likely" to use to improve their knowledge of FA; a handbook, basic course and web-based programme were the most popular choices. Results are displayed in Figure 2.

Discussion

This study set out to compare self-reported food allergy knowledge and competencies of dietitians in the UK, USA and Australia, by combining previously published data from USA –based paediatric dietitians [11] with new data from Australia and the UK. Overall we found

Table 3 Correlation between competency in food challenge protocols and feeding problems and participant characteristics

	UK (n = 336)		Australia (n = 150)	
	Food challenge protocols	**Feeding problems**	**Food challenge protocols**	**Feeding problems**
Years of practice	r = 0.12	r = 0.246	r = 0.204	r = 0.26
	p < 0.05	p < 0.01	p < 0.01	p < 0.01
Setting of practice	r = 0.08	r = 0.02	r = 0.019	r = 0.04
	p = 0.71	p = 0.32	p = 0.40	p = 0.29
Caseload of allergy patients	r = 0.32	**r = 0.50**	**r = 0.487**	**r = 0.517**
	p < 0.01	**p < 0.01**	**p < 0.01**	**p < 0.01**
Specialist allergy conference	**r = 0.423**	**r = 0.478**	r = 0.256	r = 0.339
	p < 0.01	**p < 0.01**	p < 0.01	p < 0.01
FA education/ workshop	r = 0.264	**r = 0.413**	**r = 0.416**	r = 0.382
	p < 0.01	**p < 0.01**	**p < 0.01**	p < 0.01

Strong positive correlations (r > 0.4) are in bold.

Table 4 Food allergy and intolerance training needs of UK and Australia-based dietitians

Topic	UK (%) n = 336	Australia (%) n = 150
Reactions to food additives	67	73
Pharmacological reactions (e.g. salicylates)	66	70
Oral Allergy Syndrome	62	68
Management of Irritable Bowel Syndrome	53	48
Cereal allergy	47	52
Cows' milk protein allergy	39	50
Soy allergy	46	39
Nut and seed allergy	45	42
Fish/shellfish allergy	43	38
Egg allergy	37	38
Coeliac disease	21	29
Lactose intolerance	34	14

evidence of suboptimal levels of knowledge and competency in several key food allergy aspects across all three countries.

The original questionnaire used by Groetch *et al.* [11] was developed to identify the self-reported food allergy proficiency and education needs of paediatric dietitians in the United States. Similarly, the later two questionnaires were administered to both adult and paediatric dietitians in Australia and the UK to establish a baseline of knowledge and competencies in order to advance the education and training of dietitians in the area of food allergy. This is in acknowledgement of the pivotal role dietitians play in the diagnosis and management of both adults and children with food allergy.

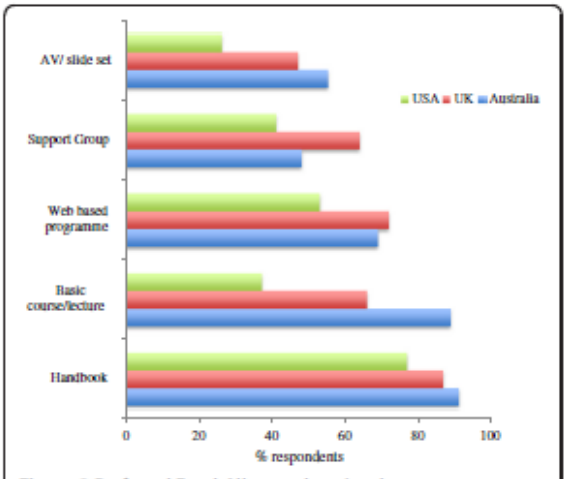

Figure 2 Preferred Food Allergy educational resources for dietitians.

Although the questionnaires were made available to dietitians working in all clinical specialities and those not working in food allergy were encouraged to respond, only 5% of the UK-based respondents reported the questionnaire was 'not at all relevant' to their practice, indicating that knowledge of food allergy is broadly relevant to the vast majority of UK-based dietitians, even if they are working in another clinical speciality. More than 50% of the Australia-based respondents were working with paediatric or adult food allergy patients at the time of the survey, again emphasising how food allergy pervades across dietetic practice. Similarly, 90% of the USA-based sample worked with food allergy patients, however this could be skewed by the fact that only paediatric dietitians were recruited in the USA and it is well known that food allergy is more prevalent in children that adults [12].

The differences seen between countries could be explained by differences in dietetic training internationally. A greater percentage of Australia and UK based dietitians than USA based dietitians, reported to have learnt about FA during basic dietetic training. Attempts have been made to standardise the undergraduate and postgraduate training of nutrition and dietetic professionals across the world [13,14]. However, a report from The International Confederation of Dietetic Associations (2008) [14] highlighted the heterogeneity of dietetic training and practice in different countries in terms of level of basic education, practical experience, competency standards and scope of practice. The importance of establishing internationalism in dietetic training in order to produce practitioners that are competent to manage emerging diseases has previously been raised [15].

A key trend emerging from these three questionnaires is the discrepancy in knowledge across different aspects of FA diagnosis and management. The public confusion that exists between perceived and actual food allergy may be contributing to this problem [16]. Although some aspects of FA management (e.g. educating patients about food avoidance, recognising signs and symptoms, understanding definitions) were well rated, others such as developing food challenges were rated poorly across all three cohorts. This was particularly the case in the UK-based cohort, where half of respondents who reported that the questionnaire was "moderately or very" relevant to their practice, rated their competency level to be "low" or "not at all proficient". This is extremely critical to the progression of allergy services in the UK, in order to ensure that patients are correctly diagnosed and timely monitored for determining tolerance to food allergens [17]. Without the availability of trained health professionals to design and implement food challenges, it is likely that patients may be incorrectly diagnosed

and placed on an exclusion diet unnecessarily. Indeed a lack of allergy services providing appropriately designed hospital-based food challenges may mean that unsafe home reintroduction challenges will be advocated, thus putting patients at risk. Reassuringly, there was a strong positive correlation between attendence at a specialist FA conference or education/workshop and competency in the area of food challenges.

Our findings are in agreement with research that has been conducted in other health professional groups across the world. A study of doctors (n = 1317) in the UK regarding knowledge of cow's milk allergy also demonstrated significant learning gaps about basic concepts [18]. Although the emphasis of the research was primary prevention of food allergy, rather than diagnosis and management, a Brazilian study of paediatricians, paediatric gastroenterologists, allergists and nutritionists (n = 520), also found gaps in knowledge across all professional groups [19]. In the USA, approximately 60% of primary care and paediatric physicians answered knowledge-based items correctly in the Chicago Food Allergy Research Survey [20]. However, only 24% were aware that oral food challenges could be used to diagnose food allergy; less than 30% felt confident to interpret biochemical results to diagnose food allergy and only 22% felt their medical training prepared them adequately to care for patients with food allergy. Finally in a South African study of dietitians and medical practitioners [21] (n = 660), 98% of respondents believed they needed more training in food allergy management at undergraduate and postgraduate level.

In our participants, although the majority of respondents used academic journals as a means to maintain CPD and some had attended food allergy conference or courses, the low number of respondents who had completed postgraduate training in food allergy should be emphasised. Further training on food additives and pharmacological reactions was requested by the UK and Australia based respondents, perhaps influenced by the adult dietitians included in both samples. In terms of resources that would be most useful, similar results were seen across the three cohorts, with a handbook, basic course or web-based programme proving most popular.

The use of online training courses has been demonstrated to be effective in increasing postgraduate knowledge in other areas of dietetics such as childhood obesity [22] and infant feeding [23]. Massive Open Online Courses (MOOCs) offer a convenient method to provide distance learning education to dietitians and health professionals internationally, with proven good completion rates and increases in competency [23]. Walsh's study [18] provides evidence of an improvement in UK doctors' knowledge of milk allergy using an online training course. Whether this success can be replicated, using a standardized approach across different countries, given the aforementioned differences in undergraduate training, remains to be seen.

There are several limitations to this study. Firstly the response rate of the questionnaires in the UK and Australia was between 3-5%, therefore it is possible that a response bias exists, where those who are interested in food allergy are most likely to participate. Each of the questionnaires was worded slightly differently, in order to adapt the content to local practices (e.g. the questionnaire used in Australia discriminated between FA and FI, the USA and UK based questionnaire did not). The UK questionnaire did not specifically ask the proportion of the caseload composed of allergy patients; instead the question of "how relevant is this questionnaire to your practice" was used as a surrogate to discriminate between those who did and did not work with patients with food allergy. In order to be more inclusive, the UK and Australia questionnaire recruited dietitians who work with both adult and paediatric patients, unlike the original USA based study, which was only aimed at paediatric dietitians. This means the results are not directly comparable. A further limitation is that all the questions were self-rated and therefore subjective. Strengths of the study design are that it included a large number of dietitians (total 797 respondents), with varied years of experience, working in different settings across three different continents.

Conclusions

There is a need amongst dietitians to increase their knowledge in different aspects of food allergy management, specifically the areas of developing food challenge protocols and management of feeding problems. Dietitians in the UK and Australia identified pharmacological reactions and food additives as the areas of greatest training need and rated a handbook, basic food allergy course or web-based programme as the most preferred methods of learning. Data from these three cohorts provides valuable information for designing food allergy education material for dietitians, which can then be adapted according to country specific needs.

Additional file

Additional file 1: Table S1. Questionnaire used in the United Kingdom.

Competing interests
The authors declare that they have no competing interests. No external funding was received to undertake this research.

Authors' contributions
KM conducted the data analysis and wrote the manuscript. RM developed the UK questionnaire and assisted with data analysis. LR developed the UK questionnaire and undertook data collection in the UK. HM designed the online questionnaire for Australia-based participants. AS, WSS and RL were

involved in design and data collection of Australia based participants. MG designed the original questionnaire, collected and analysed the USA data. CV was responsible for the overall concept, design of the UK questionnaire, initial data analysis and revision of manuscript. All authors reviewed the manuscript and approved the final version.

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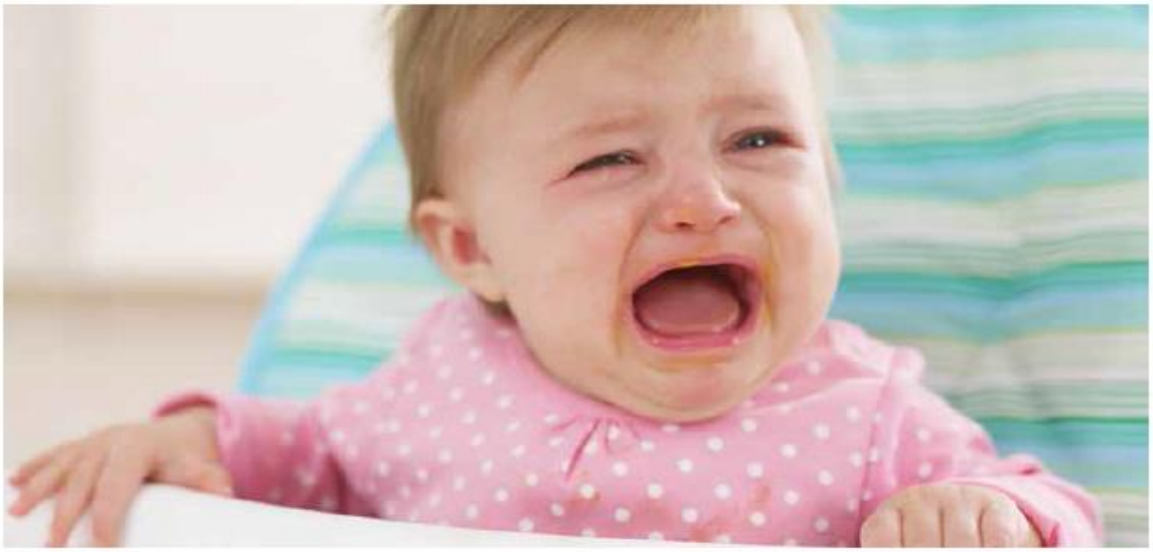
Appendix 5

Allergies

Developing a clear understanding of cow's milk protein allergy



Understanding how cow's milk protein allergy (CMPA) presents in infants is key to health visitors making an accurate initial assessment and subsequently advising on a specialist referral. Here experts **Carina Venter** and **Kate Maslin** guide you through the process

Kate Maslin *MSc RD* and **Carina Venter** *PhD RD*

According to the World Allergy Organization[1] about 1.9% to 4.9% of children suffer from cow's milk protein allergy (CMPA), yet perceived food allergy could be up to 10 times higher than that confirmed by appropriate tests[2,3]. Infants are exposed to cow's milk protein via the maternal diet if breast-fed, via standard infant formula, or when solids are introduced. It is, therefore, not surprising that cow's milk is often identified as a possible cause for skin and gut problems, particularly in early infancy[4].

The Clinical Guideline 116 on the *Diagnosis and Assessment of Food Allergy in Children and Young People in Primary Care and Community Settings*[5], produced in 2011 by the National Institute for Health and Clinical Excellence (NICE), recommends that many manifestations of food allergies could be managed in primary care. However, this necessitates health visitors having correct and up-to-date information on the different manifestations of CMPA in order to make an accurate diagnosis and referral.

Understanding how allergy works within the immune system

The first step in ensuring a satisfactory diagnosis is to have a good understanding of the immune mechanisms involved. According to the European Academy for Allergy and Clinical Immunology (EAACI) and World Allergy Organization (WAO), an adverse reaction to cow's milk can be referred to as CMPA if it involves the immune system[6]. Non-allergic cow's milk hypersensitivity (lactose intolerance), on the other hand, does not involve the immune system. CMPA is further divided into immediate (IgE-mediated) and delayed (non-IgE-mediated) allergic reactions, although some infants will present with a combination of both[7].

Key symptoms of CMPA

According to the NICE guidelines[5], food allergy can manifest as a number of different clinical

characteristics, mainly affecting the skin, gastrointestinal tract and respiratory systems, as summarised in *Box 1*.

Making an accurate diagnosis of CMPA

Although there is no official UK guidance on the diagnosis of CMPA specifically as yet, (British Society of Allergy and Clinical Immunology guidelines will be published in 2013), current NICE guidelines[5] emphasise that CMPA should be particularly considered in infants:

- Where there is a family history of allergic disease (but N.B. no family history of allergy does not exclude the possibility of becoming allergic)
- Where symptoms are persistent and affecting different organ systems
- Who have been treated for moderate to severe atopic eczema, gastro-oesophageal reflux disease (GORD) or other persisting gastrointestinal symptoms (including colic, loose stools and constipation), but have not responded to the usual initial therapeutic interventions.

History taking is essential

Taking an allergy-focused history forms the cornerstone of the diagnosis of CMPA and NICE also recommend that questions should be asked regarding:

- Any family history of atopic disease (asthma, atopic eczema, allergic rhinitis or food allergy) in parents or siblings
- Any personal history of early atopic disease (eg. atopic eczema), less commonly upper and lower airway signs, and any obvious allergic reactions to other foods
- The infant's feeding history – whether breast-fed or formula-fed – and timing of weaning (if commenced)
- Presenting symptoms and signs that may be indicating possible CMPA
- Details of previous management or dietary avoidance and any response to these interventions[5].

What is the most appropriate infant formula for CMPA?

Choosing an appropriate formula for the infant should be based on clinical presentation, nutritional composition and residual allergenicity of the formula, although the palatability and age of the infant will also be factors. It is thought that an extensively hydrolysed formula (EHF) will improve symptoms in at least 90% of infants with CMPA[8]. Therefore, use of an EHF will be the first-line treatment in most situations apart from those listed in *Box 2*. The DRACMA guidelines[3] performed a comprehensive review of the literature and indicate the use of an amino acid-based formula (AAF) for anaphylaxis, Heiner's syndrome, and eosinophilic oesophagitis, with the use of an extensively hydrolysed formula (EHF) for all other clinical presentations. Four additional papers, however, suggest the use of AAF for growth faltering, severe atopic dermatitis, multiple food allergies and infants who are not responding to maternal avoidance of cow's milk[9-12].

Box 1. Signs and symptoms of possible food allergy according to the NICE guidelines[5]

IgE-mediated (Immediate)	Non-IgE-mediated (delayed)
The skin	
• Pruritus • Erythema • Acute urticaria • Acute angioedema (most commonly in the lips and face, and around the eyes)	• Pruritus • Erythema • Atopic eczema
The gastrointestinal system	
• Angioedema of the lips, tongue and palate • Oral pruritus • Nausea • Colicky abdominal pain • Vomiting • Diarrhoea	• Gastro-oesophageal reflux disease • Loose or frequent stools • Blood and/or mucus in stools • Abdominal pain • Infantile colic • Food refusal or aversion • Constipation • Perianal redness • Pallor and tiredness • Faltering growth plus one or more of the gastrointestinal symptoms listed above (with or without significant atopic eczema)
The respiratory system (usually in combination with one or more of the above symptoms and signs)	
• Upper respiratory tract symptoms – nasal itching, sneezing, rhinorrhoea or congestion (with or without conjunctivitis)	
• Lower respiratory tract symptoms (cough, chest tightness, wheezing or shortness of breath)	
Other	
• Signs or symptoms of anaphylaxis or other systemic allergic reactions	

Confirming the diagnosis

Immediate allergic reaction (IgE-mediated CMPA)

For the diagnosis of IgE-mediated CMPA, the use of a skin prick test or specific IgE tests are recommended, but these should only be performed by those with the competencies to interpret the tests[13]. It is important to understand that a positive SPT or specific IgE test merely indicates sensitisation and does not confirm clinical allergy. However, a positive test coupled with a very clear history of a reaction may be able to confirm a diagnosis, although an oral food challenge (after a period of cow's milk avoidance) in a hospital setting will be required in many cases to confirm the diagnosis[14].

Delayed allergic reactions (Non-IgE-mediated CMPA)

There are no validated tests for the diagnosis of non-IgE-mediated CMPA, apart from an avoidance of cow's milk for four to six weeks, followed by reintroduction or a home challenge to confirm the diagnosis[14]. Home challenges may not be acceptable in children with severe forms of non-IgE-mediated cow's milk allergy, especially where food protein enterocolitis is suspected, and these children should be referred to secondary/tertiary care[5].

Maternal avoidance of cow's milk in the case of breast-fed infants, or choosing an appropriate formula for bottle-fed/partially bottle-fed infants, are crucial steps in the diagnosis of CMPA. Mothers avoiding cow's milk from their diet should be supplemented with calcium and vitamin D and ideally referred to a dietitian[15].

What is the difference between lactose intolerance and cow's milk protein allergy?

Lactose intolerance does not involve the immune system, but is caused by a deficiency of the enzyme lactase, resulting in an inability to digest lactose (milk sugar). It typically presents after a bout of gastroenteritis with symptoms of loose, watery stools, abdominal bloating and pain, increased flatus, and nappy rash, which usually improve after six to eight weeks of lactose avoidance[1]. Hard cheese and yoghurt have lower levels of lactose than cow's milk and are usually tolerated in those with lactose intolerance.

What about soya formula?

Soya-based infant formula is not recommended for infants under six months (with or without suspected CMPA) and is not the first choice treatment for infants over six months with suspected CMPA due to the risk of co-existing milk allergies and the isoflavonoid content[16]. Soya infant formula is occasionally used in infants over six months of age if EHF is unpalatable, as are soya-based weaning foods, which can be a useful source of calcium. Introduction of soya-based infant formula or weaning foods should be discussed with a paediatric dietitian.

Box 2: Clinical presentation of CMPA and choice of specialised infant formula

Clinical presentation[1]

- Acute urticaria or angioedema
- Atopic dermatitis
- Cow's milk protein-induced gastroenteritis and proctocolitis
- Constipation/diarrhoea
- Cow's milk protein-induced enteropathy
- Food protein-induced enterocolitis syndrome (FPIES)
- Gastro-oesophageal reflux disease
- Immediate gastrointestinal allergy

Extensively hydrolysed formula

Extensively hydrolysed infant formulae (EHF) available in the UK for use in infants with CMPA

Name	Manufacturer	Age	Constituents
Pepti 1	Milupa Aptamil	0–6 months	Extensively hydrolysed whey
Pepti 2	Milupa Aptamil	6–12 months	Extensively hydrolysed whey
Nutramigen Lipil 1	Mead Johnson	0–6 months	Extensively hydrolysed casein
Nutramigen Lipil 2	Mead Johnson	6–12 months	Extensively hydrolysed casein

In case of poor resolution of symptoms, choose

Clinical presentation

- Anaphylaxis[1]
- Allergic eosinophilic oesophagitis[1]
- Breast-fed infants who are not responding to maternal milk avoidance[9-12]
- Growth faltering[9-12]
- Milk-induced chronic pulmonary disease (Heiner's syndrome)[1]
- Multiple food allergies[9-12]
- Severe eczema[9-12]

Amino acid formula

Amino acid-based infant formulae (AAF) available in the UK for use in infants with CMPA

Name	Manufacturer	Age	Constituents
Neocate LCP	Nutricia	0–12 months	Amino acids
Nutramigen AA	Mead Johnson	0–12 months	Amino acids

Case Study: George

George was born at full term with a birth weight of 3.49kg (<50th centile). George's mum has a severe peanut allergy and a history of milk and egg allergy in childhood.

George was fully breast-fed but refluxing after every feed. By one month old, his weight had fallen to the 9th centile. A breastfeeding advisor suggested mum should exclude cow's milk from her diet for one week and monitor George's symptoms. George's mum found the milk-free diet difficult to follow as her diet was already restricted due to her peanut allergy. At George's six-week check, the health visitor was concerned regarding a "rash" (possibly eczema) and poor feeding; therefore George was prescribed Nutramigen and Gaviscon by his GP. No referral to the dietitian was made at this point and the mum was not advised to take any calcium or vitamin D supplements.

George was referred to the paediatrician when he was 12 weeks old as his weight had dropped to the 0.4th centile, despite fully feeding on Nutramigen. A two-week trial of Neocate was commenced after discussion with the allergy specialist dietitian and usual skin care advice given as per the NICE guidelines on eczema[17]. After two weeks on Neocate, George was vomiting less and his weight gain was starting to accelerate. Skin prick tests were negative to milk, wheat and soya, but positive to egg. The skin prick test to milk was negative, but the clinical history was strongly suggestive of CMPA.

Research suggests that milk and egg allergies often present as eczema[18], so George was given milk and egg free weaning advice by the paediatric dietitian. Two months later, George was feeding well on Neocate and consuming amounts appropriate for his weight and age. He was having a varied diet, despite avoiding milk and egg. His weight had increased to >25th centile (7.9kg) and his eczema was much improved.

It is usual practice to consider re-challenging after a period of six to 12 months of dietary exclusion. When George was reviewed by the dietitian at one year old, he had been having fromage frais for the previous two weeks without any adverse effect. He was therefore advised to follow a gradual milk reintroduction ladder at home, with firm instructions to stop immediately if there were any signs of allergy. His skin prick test to egg was negative (2mm), followed by a negative open egg challenge on the paediatric ward. Two months later, George was tolerating cow's milk so Neocate was discontinued.

Conclusion

Cow's milk protein allergy (CMPA) can present with a spectrum of immediate or delayed symptoms that can be mild, moderate or severe in nature. Symptoms may affect the respiratory and gastrointestinal systems (or a combination of these systems), and diagnosis is dependent on whether the allergy is IgE- or non-IgE-mediated in nature. Skin prick tests in isolation should be interpreted with caution by an experienced health professional, as diagnosis of allergy is dependent on a variety of factors. However, the community health professional can assist in this process by taking a thorough family history. Although the initial diagnosis and management of CMPA can take place in primary care, all infants on a cow's milk exclusion diet should ideally be referred to a paediatric dietitian, preferably before weaning onto solid food takes place. Referral to secondary care should be made as per the UK NICE guidelines[5] in more severe presentations of cow's milk allergy.

Further information:

The ACT on CMPA campaign:
Allergy UK, in partnership with infant nutrition specialists Danone Baby Nutrition and Nutricia Advanced Medical Nutrition have launched an awareness campaign – ACT on CMPA.

ACT stands for:
Awareness of the symptoms
Connect the symptoms together
Take action - could it be CMPA?

For further information visit
www.aptamilprofessional.co.uk
Aptamil Professional: 0800 996 1234

www.allergyuk.org
Allergy UK: 01322 619 898

www.anaphylaxis.org.uk
Anaphylaxis Campaign: 01252 542 029

www.neocate.co.uk
Neocate Healthcare Professional: 01225 751 098

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Appendix 6

LETTER TO THE EDITOR

Validation and acceptability of double-blind, placebo-controlled food challenges in children

To the Editor:

The double-blind, placebo-controlled food challenge (DBPCFC) is considered the gold standard for food allergy diagnosis (1, 2). It is recommended that active and placebo challenge foods for DBPCFCs are sufficiently blinded in terms of odour, taste and texture. Difficulties arise with children undergoing DBPCFCs as they may refuse to eat the challenge food or struggle to eat the large portions required to adhere to internationally recommended dosages (2).

Validated recipes for DBPCFCs have been published (3–5) using paired comparison or triangle testing to compare sensory characteristics of active and placebo foods. Only one previous study (5) has looked at the blinding of challenge foods using children as tasting panellists. While the use of adult tasting panellists may improve the quality of blinding, achieving this high level may require compromise in other aspects (e.g. larger portion sizes or use of strong flavourings). The aims of this study were to validate food challenge recipes for DBPCFCs in children and to determine the acceptability of recipes and portion sizes.

We invited children aged 5–15 years from four schools on the Isle of Wight to participate. Children were excluded if they were diagnosed with food allergy or food aversion. Recipes and challenge doses for peanut, wheat, milk and egg were calculated using international guidelines (2). For each allergen, placebo and active test doses were developed with an acceptable portion size, using optimal matrix ingredients and matching of sensory properties (3). Foods were tested in child-friendly formats; baked egg in a lemon coconut cake, peanut in chocolate biscuits, milk in a fruit mousse and wheat in chocolate cake. The blindness of recipes was tested using a triangle test. This is a forced choice procedure, whereby the taster must detect the 'odd' sample (i.e. containing the allergen or not) when given three portions of food in a randomized order (6). The foods were also compared in terms of taste, appearance and smell.

To determine whether participants could distinguish the odd sample from the other two, the critical significant number was identified using the formula $[x = (n/3)+1.64\sqrt{(2n/9)}]$ (7). We considered that they were not able to identify the odd one correctly if $p > 0.05$ (5). A two-tailed binomial exact test with the probability of 0.5 was used to determine whether participants could correctly identify if the 'odd sample' they chose contained the allergen or not.

We recruited 70 children (34 female, 36 male. Age range 5–15 years). The results of the study are summarized in Table 1. For the peanut recipe, 35 of 64 (55%) correctly identified the odd sample, indicating that the children were able to identify differences in taste, odour and texture ($p < 0.001$). A total of 26 (74%, $p = 0.03$) also correctly identified that this odd sample either contained/did not contain peanut. A total of 79% of children indicated that they liked the taste of the biscuits and could eat two. For milk, 32 of 60 (53%) correctly identified the odd sample ($p = 0.001$). Fifteen of 32 (48%, $p = 1.00$) correctly identified that this odd sample either contained/did not contain milk. A total of 48% indicated that they could eat a whole portion of the mousse. For wheat, 24 of 66 (36%) correctly identified the odd sample ($p = 0.322$), 7 of 24 children (32%, $p = 0.134$) correctly identified that this odd sample either contained/did not contain wheat. A total of 85% indicated that they could eat a whole portion. For the baked egg challenge, 24 of 61 (39%) correctly identified the odd sample ($p = 0.179$). Nine (38%, $p = 0.307$) identified that the odd sample either contained/did not contain egg correctly. A total of 36 (57%) indicated that they could eat a whole portion of the cake.

In summary, we have managed to test four separate recipes: containing peanut, milk, wheat and baked egg. We were able to validate the wheat and baked egg recipes in an acceptable portion. However, we were unable to validate the peanut and the milk recipes. Very interestingly, the ability of the children to correctly identify the odd sample as containing the allergen or not, did not correlate in all recipes with their ability to correctly identify the allergen. As expected, participants reported good acceptability for the chocolate biscuits and

Table 1 Results of triangle test and acceptability of portion size

Food	Number of children	Number of correct answers (%)	Critical number of significance	p value*	Number identified if odd one was allergen or not (%)†	p value**	Acceptability of portion size (%)
Peanut	64	35 (55)	28	0.001	26/35 (74)	0.003	50 (79)
Milk	60	32 (53)	26	0.001	15/32 (48)	1	29 (48)
Wheat	66	24 (36)	28	0.322	7/24 (32)	0.134	56 (85)
Egg	61	24 (39)	26	0.179	9/24 (38)	0.307	35 (57)

†Number of children correctly identified if the odd one had allergen or not-restricted to those who identified the odd one correctly.
*p value – one tailed binomial test with probability of 0.33 (1 in 3).
**p value – exact test.

chocolate cake, with less acceptability for the fruit mousse and the lemon coconut cake.

It has been proposed that adults rather than children should be used as tasting panellists. Performing food challenges in children can be a challenge in itself, and developing and validating food challenge recipes is expensive and labour intensive. If using panels with children, it is important that they can understand the purpose of the test, can concentrate for a sufficient amount of time and most importantly, be able to recognize and describe different kinds of taste and odour of foods (8). This study therefore highlights an important point: Can children correctly identify the taste of a specific food (e.g. milk)? It has previously been reported that 8- to 9-year-old children are able to correctly identify a taste as sweet, sour or salty when it was the only taste present, but they performed poorer than adults in correctly identifying components when there were two tastes present (9). This is confirmed by other researchers (8) who report that differences between children and adults are more likely to reveal themselves with complex (i.e. real foods) rather than simple taste stimuli; however, neither of these studies specifically tested to food allergens.

A limitation of the study was that the recipes should have been tested in stages, using adults initially, and recipes adapted accordingly. A strength of the study is that the recipes were tested in children, taking into account the international recommended challenge dosages. In conclusion, we found that testing in children with familiar allergenic foods is feasible, although the quality of the blinding may be somehow compromised compared to adults. We were able to validate food challenge recipes for children containing wheat and baked egg. We were unable to validate recipes containing peanut flour and milk. This suggests there may be a sizeable waste of resources when recipes are not blinded and emphasizes the difficulties in developing such recipes, particularly for peanut.

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Appendix 7

Allergy

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The Future of Infant and Young Children's Food: Food Supply/ Manufacturing and Human Health Challenges in the 21st Century

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Abstract

Infant food and weaning practices are highly debated with lots of unanswered questions. It is becoming more apparent that early-life feeding may have an effect on the long-term health of humans, particularly for noncommunicable diseases such as obesity and allergic diseases. It is important to understand how environmental influences in early life can affect the development of the immune system and metabolic profiling. In terms of nutrition and diet, one should consider the role of the total/whole diet, as well as particular nutrients in the development of noncommunicable diseases. Providing the appropriate nutrition for infants during the weaning age needs to address factors such as the microbial load of the food, nutrient composition, presence/absence of allergens and appropriate textures. These factors are of importance irrespective of whether the food is homemade or produced commercially, and need to take environmental factors and food resources into account.

Health Challenges

Obesity

There has been a marked increase in obesity rates over the past 20 years. Obesity and overweight affect more than 1.5 billion adults and account for 0.7–2.8% of health care costs in both the developed and developing world [1, 2].

The cost of obesity and overweight to the UK economy was estimated at GBP 15.8 billion per year in 2007, including GBP 4.2 billion in costs to the NHS [3]. In 1993, 13% of men and 16% of women were obese compared to 24% of men and 26% of women in 2011 [4]. There has also been an increase in the incidence of childhood and adolescent obesity worldwide, an important predictor of adulthood obesity, morbidity and mortality [5–7]. In the USA, a third of children were classified as either overweight or obese in 2012. The average weight of a child has risen by more than 5 kg within three decades. This varies from an increase of 2 kg in children under 8 years of age to an increase of more than 8 kg in some adolescent age groups [8]. A survey in English schoolchildren found that 19.2 and 33.9% of 4-/5- and 10-/11-year-old children, respectively, are obese or overweight [9]. However, despite an increase in obesity-related hospital admissions in children, the obesity rates of reception age children in the UK have stayed stable, albeit at a high rate of about 22%.

Maternal overweight [10–15] is associated with subsequent obesity in offspring, highlighting the importance of early-life factors in the development of obesity. In addition, increased birth weight has been associated with childhood and adolescent obesity. Data from the Isle of Wight have recently indicated [16] that an early persistent obesity was seen in obese infants but also in 'normal-weight' infants who went on to become obese teenagers/adults, i.e. some obese infants became obese adults but normal-weight infants could also become obese adults. Most importantly, this weight trajectory, also indicating the risk for developing asthma by 18 years of age, was set by 4 years. Maternal overweight before pregnancy and smoking during pregnancy were associated with increased risk in this group. The data, therefore, indicate that there are factors in early life that could lead to a 'normal-weight' infant developing into an overweight/obese infant that can persist into adulthood.

Early-Life Dietary Factors Affecting Obesity

There is some evidence indicating the microbiota is affected in obese individuals, and most importantly that microbiota as early as 3 months of age could be related to obesity at 10 years of age. This has been summarized in a review by Clarke et al. [17] (table 1). Diet, however, also affects the gut microbiota (e.g. a low fat diet is characterized by higher levels of Bacteroidetes and lower levels of Firmicutes). Weight reduction does not seem to affect gut bacteria [18] and it is therefore still unclear at this stage if the difference in gut bacteria is primarily related to body weight or diet. Early food exposure and introduction of solid food can also affect obesity outcomes, by affecting taste and food preferences to some extent.

Table 1. Gut bacteria in obesity (adapted from Clarke et al. [17])

Obese adults vs. lean adults (some conflicting data from papers)
Lower or higher levels of Bacteroidetes
Lower or higher levels of Firmicutes
Lower levels of bifidobacteria
(Some papers stating no difference)
Obese children vs. lean children
Higher levels of *S. aureus*
Lower levels of bifidobacteria
Lower levels of *Faecalibacterium prausnitzii*

Allergy

Similar to the rise in obesity, we have seen a rise in allergic diseases. The WAO White Book on allergy states that 'the prevalence of allergic diseases worldwide is rising dramatically in both developed and developing countries. These diseases include asthma; rhinitis; anaphylaxis; drug, food, and insect allergy; eczema; and urticaria (hives), and angioedema. This increase is especially problematic in children, who are bearing the greatest burden of the rising trend, which has occurred over the last two decades. In spite of this increase, even in the developed world, services for patients with allergic diseases are fragmented and far from ideal. Very few countries have comprehensive services in this field of medicine' [19]. In the USA, the overall economic cost of food allergy is estimated at USD 24.8 billion annually (USD 4,184 per year per child). This includes direct medical costs such as clinician visits, costs borne by the family (lost labor productivity due to caregiver needing time off work) and the cost of specialized foods [20]. In the UK, allergy accounts for 6% of GP consultations and 10% of the prescribing budget [21]. The direct costs of allergy to the NHS budget is GBP 1 billion per annum. This further emphasizes the need for preventing allergic diseases.

Early-Life Dietary Factors Affecting Allergy Outcomes

The role of the microbiota in the development of allergic disease has been researched for some time, with data indicating differences between the gut bacteria of allergic and nonallergic infants (table 2) [22]. This may be due to a decline in microbial exposure during early infancy, which in turn could be affected by environmental factors such as diet or early-life nutrition.

Food diversity in early life may also affect allergy outcomes. In 2013, Nwaru et al. [23] indicated that by 12 months of age, less food diversity was associated with increased risk of any asthma, atopic asthma, wheeze and allergic rhinitis. Despite some controversy and debate, micronutrients such as vitamins A, E, C

Table 2. Differences in bacterial load of infants with and without allergic disease [20]

Allergic vs. healthy infants
Less enterococci in the 1st month
Less bifidobacteria in the 1st year
Higher counts of clostridia at 3 months
Higher counts of *S. aureus* at 6 months
Lower counts of Bacteroidetes at 12 months

and D, selenium and zinc may also affect allergy outcomes, as discussed in a review by Nurmatov et al. [24] in 2011. Finally, in 2013, Grimshaw et al. [25] indicated that a diet low in commercial baby foods was associated with less food allergy in the infant.

The Infant Diet

Infant foods and the weaning diet (also referred to as introduction of solid foods) have been at the forefront of dialogues in the scientific world and media over the past few decades. Answers to questions such as the most appropriate age of introduction of solid or allergenic foods, should breastfeeding continue alongside solid food introduction, what are the crucial times for introduction of different textures, and should organic or nonorganic foods be used have been sought.

Central to all of these points is the use of homemade versus commercially available infant foods. The UK has seen an increase in baby food sales from GBP 303 million in 1995 to GBP 872 million in 2013 [26]. This is reflected in particular in the increase in sales of organic baby food. Worldwide, the baby food industry is worth USD 50 billion and is growing at a rate of 7% per year. These commercially prepared foods clearly constitute a large proportion of the infant diet, and should take into account factors that may play a role in the development of noncommunicable diseases. It is well known that association does not always equal causation. Black and Sharpe [27] published a paper looking at the association between fat intake and increase in allergic diseases in 1997. This association has never been proven in randomized controlled trials, and it will be interesting to see if there is any merit in the association seen between increase in sales of commercial baby foods and food allergy (using food-related anaphylaxis as a proxy; fig. 1). It is thought that gut microbiota has an effect on the development of allergic disease, and that certain foods and nutrients may also play a role in the prevention of allergic disease, e.g. fish/fish oil, vitamins A, E, C and

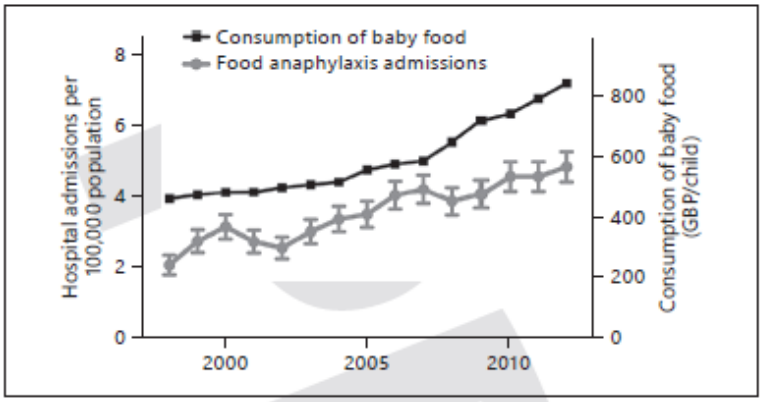

Fig. 1. Sales of commercial baby food in the UK versus admissions in food-related anaphylaxis [adapted from ref. 31].

D, selenium and zinc. Recent studies also indicate that a home-prepared [28] and more diverse diet leads to less allergic disease [29].

There are a number of ways in which the future of commercial baby foods can be improved to address the knowledge we have about possible preventative measures of allergic disease: the importance of microbes; nutrients that may play a role in the development of the immune system, and the importance of providing (some) homemade foods. The microbial load of commercially prepared baby food is negligible as food safety measures need to fulfill F_0 requirements. F_0 is a term used in the canning industry to denote the minimum process required to destroy *Clostridium botulinum* spores, which are the most deadly of all bacteria, dependent on the material being processed. However, when home-cooked food is provided to infants, up to 65% of the daily microbial load may be provided by fresh fruits and vegetables. As only a finite number of foods are included in baby foods, an increase in consumption of commercial infant foods (with corresponding decrease in consumption of home-cooked foods) may affect the diversity of foods introduced during the weaning period and ultimately affect allergy outcomes. Finally, the importance of nutrients such as antioxidants and other nutrients also require consideration when discussing commercial baby food production. It is known that sterilization can reduce the vitamin C content by up to 50% and the vitamin B_1 content by up to 30% [30].

Focus Group Results

Another very important factor to take into consideration is maternal/paternal experience of weaning and how they want the weaning message to be conveyed to them. This may not be important in terms of food production, but most baby food

manufacturers also provide information to parents of young infants. We conducted 4 × 2.5 h focus group discussions with mothers of babies 4–7 months old. Babies had either commenced weaning solids already or were about to start weaning solids. The sample of mothers was split into groups by awareness/experience of allergies, social class and whether they were a first- or second-time mother.

Three groups emerged from the analysis. These were 'practical', 'balanced' and 'anxious'. Those in the 'practical' group were confident about weaning. They were often second-time mums who perceived prepared baby food as good (if not better) than homemade baby food. Mothers in the 'balanced' group had a balanced approach to weaning. They perceived homemade baby foods as ultimately the optimal choice, but conceded that it was not always practical to prepare and feed homemade foods. These mothers tended to examine food labels closely to determine the ingredient content. Those in the 'anxious' group tended to be either first-time mums with limited knowledge or second-time mums who had heard negative stories about weaning. As a result, this group often sought advice and guidance from experts. They also perceived homemade baby food as optimal, but some thought baby food has potential to be 'safer'. Despite these three distinct typologies, all three groups had an underlying common perspective, viewing the goal of weaning as enjoyment of food and have development of a broad palate. They did not want the weaning message to be too directive, medical or pharmaceutical, and wanted the weaning message to center around 'pure', 'simple' and 'healthy'.

With regard to timing of weaning, there were two patterns. Second-time mothers tended to be 'baby led', using cues such as changes in sleep pattern, finishing milk quickly, being more irritable and watching others in the family eat. In contrast, 'advice-led' mothers sought advice from health care professionals, the internet, books, their own mothers or friends. Those in the higher social class were more likely to seek advice from health care professionals. Besides timing of weaning, the process of weaning (e.g. risk of choking) and what food to give were other sources of worry. For the majority of mothers these concerns were very short lived. Once weaning had started concerns over when and how were almost instantly overcome. However, concerns over what to feed and how quickly to introduce new flavors and foods into the diet were more variable between mothers.

Generally, mothers commenced the weaning process with excitement and good intention of cooking homemade foods. However, even those who did a lot of home cooking reported that preparing fruit purees could be 'hassle'. It was felt that prepared baby foods were composed of simple, safe ingredients. Indeed they were viewed by some to be superior to homemade foods especially if they were organic, prepared by better cooks and used better ingredients.

The choice of what prepared baby food to use was driven by three key factors: 'taste', 'goodness' and 'the truth'. 'Taste' was characterized mainly by the description of the ingredients and recipes. 'Goodness' helped mothers decide whether the product was healthy. Finally, mothers were keen to know the 'truth' about prepared baby foods (i.e. what exactly was contained in the food and what was hidden). Specifically, they were keen to discover whether the products contained milk, eggs, gluten and nuts, in addition to preservatives, coloring and salt. Interestingly, there was very little spontaneous mention of food allergies. When probed, mothers in the 'balanced' and 'anxious' groups showed some concerns, but the vast majority thought that food allergies are very individual and that exposure to a variety of foods early in the weaning process was important to identify any issues.

Conclusion

There is no doubt that the weaning diet will affect later health outcomes alongside maternal eating practices during pregnancy and breastfeeding as well as early milk (breast milk or formula) consumption. This poses the opportunity for families cooking homemade foods and industry producing commercial baby foods to provide infant foods in order to (perhaps) stem the tide of noncommunicable diseases such as allergy and obesity. Therefore, in the future, the introduction of food to infants should focus on three main factors:

(1) Parental cooking skills to provide freshly cooked, homemade food

(2) The possible bacterial content of commercial (sterilized vs. pasteurized) versus home-made foods

(3) The particular nutrient content of baby foods and diversity of the infant diet.

This will have to be provided with the backdrop of current dwindling world resources, focusing specifically on the availability and sustainable production of fish and meat, better food distribution and less food waste at home.

Disclosure Statement

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Appendix 8: Literature Search Strategy

The following electronic databases were searched: Pubmed, Science Direct, CINAHL, Zetoc, Cochrane library.

Conference proceedings and abstracts from the American Academy of Allergy and Clinical Immunology (2012-2015) and European Academy of Allergy and Clinical Immunology (2012-2015) were searched.

The following keywords and synonyms were identified using the Patient Intervention Comparison Outcome (PICO) approach. Search terms were combined using Boolean operators.

Population/ Patient	**Intervention or Exposure**	**Comparison**	**Outcomes**
Infant Child*	Cows' milk allergy Extensively hydrolysed formula	Formula fed	Eating habits Fussy eating Taste preferences Food preferences Food neophobia Growth Dietary intake Nutritional intake Feeding difficulties
Alternative search terms	**Alternative search terms**	**Alternative search terms**	**Alternative search terms**
Baby	Specialised infant formula Amino acid infant formula Hypoallergenic infant formula Milk free diet Exclusion diet	Breastfed	Picky eating Faddy eating Selective eating Food likes Food acceptance Feeding problems Food aversion Weight gain

Relevant citations and key authors were identified. Hand searching of reference lists was undertaken. To prevent bias, no restriction was placed on the year of publication or language. CASP (critical appraisal skills programme) was used to appraise identified literature.

Appendix 9: Children's Eating Behaviour Questionnaire

Please rate each of the following statements on a scale of 1 (never) to 5 (always) by circling one number

	Never				Always
a. My child enjoys eating	1	2	3	4	5
b. My child loves food	1	2	3	4	5
c. My child is interested in food	1	2	3	4	5
d. My child looks forward to mealtimes	1	2	3	4	5
e. My child gets full up easily	1	2	3	4	5
f. My child has a big appetite	1	2	3	4	5
g. My child leaves food on his/her plate at the end of a meal	1	2	3	4	5
h. My child gets full before his her meal is finished	1	2	3	4	5
i. My child cannot eat a meal if he/she has have a snack just before	1	2	3	4	5
j. My child eats slowly	1	2	3	4	5
k. My child takes more than 30 minutes to finish a meal	1	2	3	4	5
l. My child eats more and more slowly during the course of a meal	1	2	3	4	5
m. My child enjoys tasting new food	1	2	3	4	5
n. My child enjoys a wide variety of foods	1	2	3	4	5
o. My child is interested in tasting foods s/he has not tasted before	1	2	3	4	5
p. My child refuses new food at first	1	2	3	4	5
q. My child decides that he/she does not like food even without tasting	1	2	3	4	5
r. My child is difficult to please with meals	1	2	3	4	5
s. My child is always asking for food	1	2	3	4	5
t. If given the chance my child would always have food in his/her mouth	1	2	3	4	5
u. Given the choice, my child would eat most of the time	1	2	3	4	5
v. If allowed to, my child would eat too much	1	2	3	4	5
w. Even if my child is full up, s/he finds room to eat his/her favourite food.					

Appendix 10: Picky Eating Questionnaire

Please circle one option to each question on the scale of 1-7

1. To what extent does your child's eating behaviour bother you?	1 2 3 4 5 6 7 Not at all To a great extent
2. To what extent would you consider your child to have a feeding problem?	1 2 3 4 5 6 7 Not at all To a great extent
3. Overall to what extent does your child like a wide variety of foods from those that you think he/she should eat?	1 2 3 4 5 6 7 Not at all To a great extent
4. Rank your child's eating behaviour as a whole	1 2 3 4 5 6 7 Extremely poor Extremely good
5. In general, at the end of the meal how often has your child eaten the amount you think he/she should eat?	1 2 3 4 5 6 7 Never Always
6. How often do you attempt to persuade your child to eat a food?	1 2 3 4 5 6 7 Never Always
7. How often do you provide a food reward for eating a food you think your child should eat ?	1 2 3 4 5 6 7 Never Always
8. How often do you prepare a special food for your child because she/he does not like what the rest of the family is eating?	1 2 3 4 5 6 7 Never Always
9. How often does your child try new and unfamiliar foods at home?	1 2 3 4 5 6 7 Never Always
10. How willing is your child to try new and unfamiliar food when offered?	1 2 3 4 5 6 7 Never Extremely willing

Appendix 11: Children's Food Neophobia Scale

We would like to know how your child reacts to new foods. Please answer each question by circling one answer on a scale from 1 (disagree strongly) to 7 (strongly agree).

1. My child constantly samples new and different foods	1 2 3 4 5 6 7 Disagree strongly Strongly Agree
2. My child does not trust new foods	1 2 3 4 5 6 7 Disagree strongly Strongly Agree
3. If my child doesn't know what is in a food, he/she won't try it	1 2 3 4 5 6 7 Disagree strongly Strongly Agree
4. My child likes foods from different countries	1 2 3 4 5 6 7 Disagree strongly Strongly Agree
5. My child thinks some foods look too strange to eat	1 2 3 4 5 6 7 Disagree strongly Strongly Agree
6. At parties, my child will try a new food	1 2 3 4 5 6 7 Disagree strongly Strongly Agree
7. My child is afraid to eat things he/she has never eaten before	1 2 3 4 5 6 7 Disagree strongly Strongly Agree
8. My child is very particular about the foods he/she will eat	1 2 3 4 5 6 7 Disagree strongly Strongly Agree
9. My child will eat almost anything	1 2 3 4 5 6 7 Disagree strongly Strongly Agree
10. My child likes to try new foreign foods	1 2 3 4 5 6 7 Disagree strongly Strongly Agree

Appendix 12: The Montreal Children's Hospital Feeding Scale

We would like to know about any feeding difficulties your child has. Please circle one number for each question on the scale of 1-7.

1. How do you find mealtimes with your child?	1 Very difficult	2	3	4	5	6	7 Easy
2. How worried are you about your child's eating?	1 Not worried	2	3	4	5	6	7 Very worried
3. How much appetite (hunger) does your child have?	1 Never hungry	2	3	4	5	6	7 Good appetite
4. When does your child start refusing to eat during mealtimes	1 At the beginning	2	3	4	5	6	7 At the end
5. How long do mealtimes take for your child (in minutes)?	1 1-10	2 11-20	3 21-30	4 31-40	5 41-50	6 51-60	7 > 60 min
6. How does your child behave during mealtimes?	1 Behaves well	2	3	4	5	6	7 Makes a big fuss
7. Does your child gag or spit or vomit with certain types of food?	1 Never	2	3	4	5	6	7 Most of the time
8. Does your child hold food in his/her mouth without swallowing it?	1 Most of the time	2	3	4	5	6	7 Never
9. Do you have to follow your child around or use distractions (toys, TV) so that your child will eat?	1 Never	2	3	4	5	6	7 Most of the time
10. Do you have to force your child to eat or drink?	1 Most of the time	2	3	4	5	6	7 Never
11. How are your child's chewing (or sucking) abilities?	1 Good	2	3	4	5	6	7 Very poor
12. How do you find your child's growth?	1 Growing poorly	2	3	4	5	6	7 Growing well
13. How does your child's feeding influence your relationship with him/her?	1 Very negatively	2	3	4	5	6	7 Not at all
14. How does your child's feeding influence your family relationships?	1 Not at all	2	3	4	5	6	7 Very negatively

Appendix 13 : Food frequency questionnaire adapted from the Southampton Women's Study

FOOD FREQUENCY QUESTIONNAIRE

I am going to ask you about the **food and drinks** your child has eaten in the **past 4 weeks**. I will ask you how often he/she has eaten certain foods and drinks. You should only include food and drinks actually eaten, do not include food and drinks that was left over or spilled. Your baby may sometimes be fed by a relative, friend or someone else. If you know the type of food and drinks and eaten at these times please include them.

MILK/MILK SUBSTITUTE/FORMULA AND OTHER DRINKS

Can you tell me the types of milk or formula he/she has had in the past 4 weeks? How many days out of the past 4 weeks (28 days) was *type of milk* given?
What was the average amount of *type of milk* **per day** on these days?

Type of milk or formula	Number of days in the past 28	Total volume per day in oz or mls

Thinking about the past month, please tick how often the child has had the following drinks:

Drinks	**Never**	**1-3 per month**	**Number of times per week**							**More than once a day**
			1	**2**	**3**	**4**	**5**	**6**	**7**	
Baby juices										
Pure fruit juice										
Fruit drinks										
Ribena, or high juice squash										
Squash, not including low calorie										
Low calorie squash										
Fizzy drinks, not including low calorie										
Low calorie fizzy drinks										
Tea										
Water										

Please indicate how often your child has eaten the following READYMADE BABY FOODS in the PAST MONTH

Readymade baby foods	Never	1-3 per month	Number of times per week							More than once a day
			1	2	3	4	5	6	7	
Dried baby cereals										
Dried meat or fish based meals										
Dried vegetable, pasta or rice based meals										
Dried desserts										
Breakfast meals such as porridge										
Meat or fish based meals										
Vegetable, pasta or rice based savoury meals										
Milk or cereal based desserts										
Fruit based desserts, not including pure fruit puree										
Pure fruit puree										

Please indicate how often your child has eating the following CEREAL BASED FOODS in the PAST MONTH

Cereal based foods	Never	1-3 per month	Number of times per week							More than once a day
			1	2	3	4	5	6	7	
White bread										
Brown and wholemeal bread										
Crackers, breadsticks, rice cakes, baby crisps										
Breakfast cereals and porridge										

Boiled and baked potatoes										
Chips, potato shapes and roast potatoes										
Rice										
Pasta										

Please indicate how often your child has eaten the following DAIRY EGG and SUBTITTUTE foods in the PAST MONTH

Dairy, eggs and substitutes	**Never**	**1-3 per month**	**Number of times per week**							**More than once a day**
			1	**2**	**3**	**4**	**5**	**6**	**7**	
Cheese										
Soya cheese										
Savoury white sauce										
Yoghurt and fromage frais										
Soya yoghurt										
Other milk substitute yoghurt (e.g. rice/coconut based yoghurt)										
Pizza										
Quiche and savoury flan										
Eggs										

Please indicate how often your child has eaten the following MEAT, FISH and VEGETARIAN SUBSTITUTE foods in the PAST MONTH

Meat, fish and vegetarian substitutes	**Never**	**1-3 per month**	**Number of times per week**							**More than once a day**
			1	**2**	**3**	**4**	**5**	**6**	**7**	
Chicken or turkey in batter or breadcrumbs										
Beefburgers										
Bacon and gammon										
Sausages										
Meat casseroles, stews, and curries										
Roast, grilled or fried meat										
Liver, kidney and faggots										

Meat pies and sausage rolls										
Ham and processed cold meats										
Fish in batter or breadcrumbs										
Other white fish										
Oily fish										
Vegetarian burgers, sausages and nuggets										

Please indicate how often your child has eaten the following FRUITS AND VEGETABLES in the PAST MONTH

Fruits and vegetables	Never	1-3 per month	Number of times per week							More than once a day
			1	2	3	4	5	6	7	
Peas										
Carrots										
Green beans										
Sweetcorn										
Sweet potato/butternut squash										
Broccoli, cabbage or brussels sprouts										
Cauliflower										
Tomatoes										
Green salad or cucumber										
Beans and pulses										
Tinned fruit										
Apples										
Bananas										
Oranges /satsumas										
Peaches or nectarines										
Strawberries,/raspberries/blueberries										
Plum										
Pear										
Melon										
Grapes										
Mango										
Kiwi										
Cherries										

Please indicate how often your child has eaten the following SWEET AND MISCELLANEOUS FOODS in the PAST MONTH

Sweet and miscellaneous foods	Never	1-3 per month	Number of times per week							More than once a day
			1	2	3	4	5	6	7	
Ice-cream										
Custard										
Other readymade desserts										
Other puddings										
Cakes, buns and pastries										
Chocolate and digestive biscuits										
Other biscuits										
Chocolate										
Sweets										
Crisps and savoury snacks										
Marmite /Bovril										
Peanut butter										
Jam/sweet spread										
Butter and margarine										
Sugar										

ADDITIONAL FOODS

If there anything else he/she has had to eat or drink 4 or more times (that is, about once a week or more) in the past 4 weeks that we have not already included, please can you list them in the table below.

Brand/Description	Number of times per week							More than once a day
	1	2	3	4	5	6	7	

Appendix 14: Infant feeding and allergy history questionnaire (infant/toddler study)
Date_____ Study number_____

Please complete this form by ticking the appropriate boxes. Please answer every question.

Child's name		Child's Date of Birth	
Child's Address		Child's GP Name & Address	
Parent/Guardian name		Parent/Guardian phone number	
Email Address			

Please indicate who is completing the questionnaire:

Researcher ☐ Mother ☐ Father □ Grandparent ☐ Guardian ☐

Other □_____

Part 1 : General information about your child and your family

1.1 Your child's gender: Female ☐ Male ☐

1.2 What is your child's ethnic background? *Please tick only one option.*

White British ☐ White European ☐ White Other ☐

Black: British ☐ Black Caribbean ☐ Black African ☐

Black Other ☐ Asian: Indian ☐ Asian: Pakistani ☐

Asian: Bangladeshi ☐ Asian: Other ☐ Mixed Race ☐

Chinese ☐ Other ☐

1.3 What is the child's parents' occupational status?
Please tick only one for each parent.

Mother		**Father**	
Student	☐	Student	☐
Self-employed	☐	Self-employed	☐
Full-time employed	☐	Full-time employed	☐
Part-time employed	☐	Part-time employed	☐
Retired	☐	Retired	☐
Unemployed	☐	Unemployed	☐
Other:	☐	Other:	☐

1.4 What is the highest qualification the child's mother and father hold?
Please tick only one for each parent.

Mother		**Father**	
None	☐	None	☐
GCSE (or equivalent qualification)	☐	GCSE (or equivalent qualification)	☐
A-level (or equivalent qualification)	☐	A-level (or equivalent qualification)	☐
Graduate level qualification	☐	Graduate level qualification	☐
Post-graduate qualification	☐	Post-graduate qualification	☐

Part 2. Family History of Allergy

2.1 Has any of the following persons ever had asthma?

MOTHER		**FATHER**		**ANY SIBLING OF CHILD**	
YES	☐	YES	☐	YES	☐
NO	☐	NO	☐	NO	☐
DON'T KNOW	☐	DON'T KNOW	☐	DON'T KNOW	☐
				NOT APPLICABLE	☐

2.2 Has any of the following persons ever had hayfever?

MOTHER		**FATHER**		**ANY SIBLING OF CHILD**	
YES	☐	YES	☐	YES	☐
NO	☐	NO	☐	NO	☐
DON'T KNOW	☐	DON'T KNOW	☐	DON'T KNOW	☐
				NOT APPLICABLE	☐

2.3 Has any of the following persons ever had an itchy rash which was coming and going for at least six months?

MOTHER		**FATHER**		**ANY SIBLING OF CHILD**	
YES	☐	YES	☐	YES	☐
NO	☐	NO	☐	NO	☐
DON'T KNOW	☐	DON'T KNOW	☐	DON'T KNOW	☐
				NOT APPLICABLE	☐

2.4 Has any of the following persons ever had wheezing or whistling in the chest at any time in the past?

MOTHER		FATHER		ANY SIBLING OF CHILD	
YES	☐	YES	☐	YES	☐
NO	☐	NO	☐	NO	☐
DON'T KNOW	☐	DON'T KNOW	☐	DON'T KNOW	☐
				NOT APPLICABLE	☐

2.5 Has any of the following persons ever suffered from an itchy, stuffy or runny nose and/or swollen, itchy eyes when they did not have a cold?

MOTHER		FATHER		ANY SIBLING OF CHILD	
YES	☐	YES	☐	YES	☐
NO	☐	NO	☐	NO	☐
DON'T KNOW	☐	DON'T KNOW	☐	DON'T KNOW	☐
				NOT APPLICABLE	☐

2.6 Has the mother of the child ever suffered from symptoms of food allergy or intolerance?

YES ☐ NO ☐ DON'T KNOW ☐

2.7 Has the father of the child ever suffered from symptoms of food allergy or intolerance?

YES ☐ NO ☐ DON'T KNOW ☐

2.9 Has any sibling of the child ever suffered from symptoms of food allergy or intolerance?

YES ☐ NO ☐ DON'T KNOW ☐

NOT APPLICABLE ☐

<u>Part 3: Questions about Child's Allergy History</u>

3.1 Has the child ever had wheezing or whistling in the chest ?

YES ☐ NO ☐ DON'T KNOW ☐

3.2 Has the child had a dry cough at night, apart from the cough associated with a cold or a chest infection?

YES ☐ NO ☐ DON'T KNOW ☐

3.3 Has the child ever had an itchy rash that was coming and going ?

YES ☐ NO ☐ DON'T KNOW ☐

3.31 Did you identify a cause for this itchy rash ?

YES ☐ Please state

_____

NO ☐ NOT APPLICABLE ☐

3.4 Has the child ever suffered from vomiting (> 1 tablespoon)?

YES ☐ NO ☐ DON'T KNOW ☐

3.4.1 Did you identify a cause for this vomiting ?

YES ☐ Please state _____

NO ☐ NOT APPLICABLE ☐

3.5 Has the child ever suffered from diarrhoea ?

YES ☐ NO ☐ DON'T KNOW ☐

3.5.1 Did you identify a cause for this diarrhoea ?

YES ☐ Please state _____

NO ☐ NOT APPLICABLE ☐

3.6 Has the child ever suffered from constipation ?

YES ☐ NO ☐ DON'T KNOW ☐

3.6.1 Did you identify a cause for this constipation ?

YES ☐ Please state _____

NO ☐ NOT APPLICABLE ☐

3.7 Has the child ever suffered from abdominal distension (bloating) ?

YES ☐ NO ☐ DON'T KNOW ☐

3.7.1 Did you identify a cause for this abdominal distension (bloating) ?

YES ☐ Please state _____

NO ☐ NOT APPLICABLE ☐

3.8 Has the child ever suffered from colic/tummy ache ?

YES ☐ NO ☐ DON'T KNOW ☐

3.8.1 Did you identify a cause for this colic/tummy ache ?

YES ☐ Please state _____

NO ☐ NOT APPLICABLE ☐

3.9 Has the child ever suffered from any other food related problems ?

YES ☐ Please state problem_____

NO ☐ DON'T KNOW ☐

3.9.1 Did you identify a cause for this food related problem ?

YES ☐ Please state _____

NO ☐ NOT APPLICABLE ☐

3.10 Are any foods currently being excluded from the child's diet because of food allergy? YES ☐ NO ☐

3.10.1 If yes, which foods, since when and why?

Food	**Age excluded (in weeks)**	**Reason**

3.11 Are any food currently being excluded from the child's diet for any other reason? YES ☐ NO ☐

3.11.1 If yes, which foods, since when and why?

Food	**Age excluded (in weeks)**	**Reason**

<u>Part 4 Child's growth</u>

4.1 Was the child born at full term?

Yes ☐ No ☐ please state gestation in weeks_____

4.2 What was the child's birthweight?

Please state_____ Don't know ☐

4.3 Please fill in any growth measurements you have from the child's red book

Date	Age	Weight	Length

4.4 Most recent weight _____ **Date**_____

4.5 Most recent Length _____ **Date** _____

4.6 Most recent head circumference_____**Date**_____

Part 5: Feeding history

5.1 Is the child currently having any breastfeeds? Yes ☐ No ☐

5.2 Was the child breastfed at all Yes ☐ No ☐

IF yes, for how long? *Please tick one box*

Up to 1 month ☐ Up to 2 months ☐ Up to 3 months ☐

Up to 4 months ☐ Up to 5 months ☐ Up to 6 months ☐

Up to 9 months ☐ Up to 12 months ☐ 12 months or more ☐

Don't know ☐

5.3 Is the child currently having any formula feeds? Yes ☐ No ☐

5.4 Was your child given formula milk at any point ?

(either as a top up OR as the baby's main drink) Yes ☐ No ☐

IF yes, when were they first given formula milk? *Please tick one box*

Before 1 month ☐ Between 1-2 months ☐ Between 2-3months ☐

Between 3-4 months ☐ Between 4-5 months ☐ Between 5-6 months ☐

Between 6-9 months ☐ Between 9-12 months ☐ After 12 months ☐

Don't know ☐

5.5 If the child was given formula milk, which ones?

Name of formula milk	How long for approximately

5.6 At what age was the child first given solid food? _____weeks old
If not sure, please could you estimate age in weeks_____

5.7 At what age was the child first given lumpy food?_____weeks old
If not sure, please could you estimate age in weeks_____

Not yet had lumpy foods ☐

5.8 At what age was the child first given finger foods? _____weeks old
If not sure, please could you estimate age in weeks_____

Not yet had finger foods ☐

5.9 What were the first foods that were introduced to the child?

Food 1_____

Food 2_____

Food 3_____

Food 4_____

5.10 Was the child predominantly weaned onto homemade foods or pre prepared baby food ? Homemade ☐ Pre prepared ☐ Mixture of both ☐

5.11 What type of diet does the child eat? Normal ☐ Vegetarian ☐ Vegan ☐

Other ☐ please state_____

5.12 Does the child currently have any medical conditions that affect their diet (e.g. diabetes)?

Yes ☐ please state _____No ☐

5.13 Does the child currently take any dietary supplements (e.g. vitamins)?

Yes ☐ please state _____No ☐

5.14 Has the child ever been seen by a dietitian?

Yes ☐ please state how many times they have been seen_____No ☐

5.15 If yes, for what reason?

Food allergy ☐ Other reason, ☐ please state _____

5.16 How much attention do you pay to the child's diet in terms of healthy eating?
Please tick one box.

Very little ☐ Somewhat ☐ A great deal ☐

Appendix 15: Ethical approval letter from Berkshire NHS Research Ethics Committee

NRES Committee South Central - Berkshire

Bristol REC Centre Whitefriars Level 3, Block B Lewins Mead Bristol BS1 2NT

Telephone: 0117 3421389 Facsimile: 0117 3420445

NHS
Health Research Authority

09 May 2013

Dear Dr Venter,

REC reference: IRAS project ID:The effect of a cow's milk exclusion diet and substitute formula in infancy on childhood eating habits. 13/SC/0194 119986

Thank you for your letter of 01 May 2013. I can confirm the REC has received the documents listed below and that these comply with the approval conditions detailed in our letter dated 30 April 2013. The documents received were as follows:

Document	Version	Date
Covering Letter		01 May 2013
Other: Protocol Front Page	1.1	01 May 2013
Participant Information Sheet: Part 1: Parents of Children from FAIR Study	1.1	01 May 2013
Participant Information Sheet: Part 1 of Study: Parents of Children Recruited through NHS Records	1.1	01 May 2013
Participant Information Sheet: Part 2 of Study	1.1	01 May 2013
Questionnaire: Validated: Southampton Food Frequency	1.1	01 May 2013

You should ensure that the sponsor has a copy of the final documentation for the study. It is the sponsor's responsibility to ensure that the documentation is made available to R&D offices at all participating sites.

Yours sincerely,

Ms Rae Granville Committee Co-ordinator

Appendix 16: Isle of Wight NHS trust research and development approval letter

RM&G Office
Planned Clinical Directorate
St Mary's Hospital
Newport
Isle of Wight
PO30 5TG

Direct Tel No (01983) 552354
Direct Fax No (01983) 552521
Email: alexandra.punter@iow.nhs.uk

~~4 July 2013~~ **Reissued 17 July 2013**

Dr Carina Venter
NIHR Senior Research Fellow, University of Portsmouth/
Senior Allergy Dietitian
Isle of Wight NHS Trust
David Hide Asthma and Allergy Centre
St. Mary's Hospital

Dear Carina

CSP 119986 : The correlation between the effect of a cow's milk exclusion diet and substitute formula in infancy and later childhood eating habits

I am writing formally to confirm research governance approval to the above project, following completion of local governance checks within the National Institute for Health Research's Co-Ordinated System for Gaining NHS Permission (NIHR CSP).

I confirm that the Isle of Wight NHS Trust has accepted the role of Sponsor for the above study, as set out in the Research Governance Framework for Health and Social Care (second edition, April 2005).

We note that NRES Committee South Central - Berkshire has granted ethical approval, which applies to all NHS sites taking part in the study. Site-Specific Assessment at NHS sites is the responsibility of NHS R&D offices and, having reviewed the documentation submitted for this project, I confirm the R&D Committee has undertaken a favourable site specific assessment of the suitability of you as Chief Investigator and your facilities.

In accordance with our Trust Policy for R&D, I draw your particular attention to the following:

- In the event of a serious adverse event, which is linked to your research study, you must report any occurrence using the Trust's Incident Reporting Procedure.

- You will be required to provide a periodic report of progress with your research to the R&D Committee. Such progress reports should include details on any research outputs as well as current participant numbers, project start and end dates and account for all research income and expenditure.

As this study has been adopted onto the UKCRN Clinical Research Portfolio, we take this opportunity to remind you of your responsibility for uploading accrual data for our organisation once we become a participating site.
(http://www.ukcrn.org.uk/index/clinical/portfolio_new/P_accrual.html)

I wish you every success with your study.

Yours sincerely

Alexandra Punter
Research Management and Governance Manager
Isle of Wight NHS Trust

cc: Kate Maslin, Research Dietitian & PhD Student, Isle of Wight NHS Trust

Appendix 17: Parents information sheet (infant/toddler study)

The David Hide Asthma & Allergy Research Centre
St. Mary's Hospital
Isle of Wight
PO30 5TP

Direct Tel. No. (01983) 534373
Email: kate.maslin@port.ac.uk

Eating Habits in Infants and Toddlers with and without Food Allergy

Information Sheet

Dear Parent/Guardian,

My name is Kate Maslin. I am an allergy dietitian working at the David Hide Asthma & Allergy Research Centre and a PhD student at the University of Portsmouth.

We would like to thank you for taking the time to read this study information sheet. We would like to invite you to help us with this study of infants' and toddlers' eating habits. Before you make any decision about involvement in the study it is important for you to understand why the research is being done and what it will involve.

Please read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear, or if you would like more information, by contacting us on the number or email address provided. Take time to decide whether or not you wish to take part.

What is the purpose of the study?
The purpose of this study is to assess eating habits in infants and toddlers with and without a food allergy. We know from previous research that fussy eating is common in this age group and that it may be more common in children with food allergies. Therefore we would like to compare a group of infants who are eating a normal diet, with a group of infants who are eating a special diet because of a food allergy.

Why has my child been chosen?
The study involves infants from the Isle of Wight between the ages of 8-30 months old; a group who have a food allergy and a group who do not have a food allergy.

Does my child have to take part?

It is up to you to decide whether or not to take part. If you decide to take part we suggest that you keep this information sheet. Please could you return the consent form indicating your willingness to take part, in the prepaid envelope provided. You and your child are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect any medical care your child may receive in future.

How is the study conducted?
The study will compare two groups of infants; one group who are eating a special diet because of food allergy and one group who are eating a normal diet.

What will happen if we decide to take part?
If you decide to take part in the study we would ask you to do the following:

Part (i) To complete some questionnaires about your child's feeding history and diet. This will take approximately 20 minutes. We will arrange an appointment for you to attend the allergy centre where the dietitian will complete the questionnaires with you in person. If this is not convenient, you can complete the questionnaires online or complete a paper copy and return to us by post.

Part (ii) To have your child's weight, length and head circumference measured. If it is not possible for your child to have these measurements done at the allergy centre, we will ask you for recent measurements from your child's red book.

What are the benefits of taking part?
Half of the infants participating in the study will be healthy volunteers. Half of the children will have a food allergy and may have eczema. The study does not include any treatment for any condition. However, your GP will be informed of your child's participation if any medical treatment is required or any nutritional issues are highlighted.

What are the possible disadvantages and risks of taking part?
There are no disadvantages or risks in taking part in this study. The only issue is the inconvenience of completing some questionnaires.

What if there is a problem?
If you have any questions or concerns, please contact Kate Maslin, Allergy Dietitian, at The David Hide Asthma and Allergy Research Centre, St Mary's Hospital, Newport, Isle of Wight. Telephone: 01983 534373. Email: kate.maslin@port.ac.uk. If you still have questions or concerns, contact Dr. Carina Venter, The David Hide Asthma and Allergy Research Centre, St Mary's Hospital, Newport, Isle of Wight. Telephone: 01983 534373. Email:carina.venter@port.ac.uk. or you can contact Alexandra Punter (Lead for Research and Development, St Mary's Hospital, Newport, Isle of Wight; Email: alexandra.punter@iow.nhs.uk).

In the highly unlikely event that something does go wrong and you are harmed during the research study there are no special compensation arrangements. If you are harmed and this is due to someone's negligence then you may have grounds for a legal action for compensation against St Mary's Hospital but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you.

Will my child get paid for his/her participation?

We will provide a small gift token to your child as a thank you for taking part. We will reimburse any travel expenses incurred by taking part.

Will my child's taking part in the study be kept confidential?
Yes. All the information about your child's participation in this study will be kept confidential. Only the study personnel will have access to your child's personal details. Your child will not be individually identified in any reports or publications resulting from the study. We will keep your child's data on file for use in future studies approved by the Research Ethics Committee. If you are at serious risk of harming yourself or others; or there are concerns for the neglect or abuse of children then we will have to share your information with agencies, this may be without your permission. If this happens we would discuss it with your first.

What will happen to the results of the research study?
We aim to publish the results of the study in medical journals so that other doctors and researchers can use our data and provide better care for their patients.

Who is organising and funding the research?
The researchers at The David Hide Asthma and Allergy Research Centre and the University of Portsmouth are organising and carrying out this study. The study is being supported by the National Institute of Health Research (NIHR) and Danone Baby Nutrition.

Who has reviewed the study?
The study has been reviewed by the Berkshire Research Ethics Committee on 9th May 2013 and was given a favourable ethical opinion for conduct in the NHS.

How long do I have to decide whether my child should take part?
Your decision to participate in this study is entirely voluntary. You should take as much time as you need, but we do ask you to let us know of your participation within the next three weeks.

What do I do now?
If you are interested in joining the study, please sign the enclosed consent form and return it to me in the enclosed envelope. I will then contact you to arrange a convenient appointment time for you to attend the allergy centre. To help me do this and if you are happy to, could you please write your phone number and email address on the consent form.

If you would prefer to complete the questionnaires online or by post, please indicate this on the consent form and they can be posted or emailed to you. If you have any questions at all then please do not hesitate to contact me and I will be happy to speak to you.

Thank you for your time

Kate Maslin & Carina Venter
Allergy Dietitians

Appendix 18:
Consent form (infant/toddler study)

The
David Hide Asthma & Allergy Research Centre
St. Mary's Hospital
Newport
Isle of Wight
PO30 5TG
Direct Tel: (01983) 534178
Direct Fax: (01983) 534373
Email: kate.maslin@port.ac.uk

Parent/Guardian Consent Form

Eating Habits in Infants and Toddlers with and without Food Allergy

Name of Researcher: Kate Maslin

Please initial box

I confirm that I have read and understand the information sheet dated 1st May 2013 (version 1.1) for the above study. ☐

I have now had the opportunity to consider the information, regarding the study, ask questions and have had these answered satisfactorily. ☐

I understand that my child's participation is voluntary and that my child is free to withdraw at any time without giving any reason, without her/his medical care or legal rights being affected. ☐

I understand that relevant sections of my child's medical notes and data collected during the study, may be looked at by individuals from the Isle of Wight NHS Primary Care Trust or from regulatory authorities where it is relevant to my child's taking part in this research. ☐

I give permission for these individuals to have access to his/her records. ☐

I understand that my GP will only be informed of my child's participation in the study, if my child requires medical treatment or any nutritional concerns are highlighted. ☐

I consent to completing questionnaires about my child's eating habits ☐

I consent to my child having their weight, length and head circumference measured ☐

_____ _____ _____
Parent/Guardian Name (in block letters) Signature Date

_____
Child's Name

_____ _____ _____
Researcher Name Signature Date

I am happy for you to contact me by:

Phone_____ ☐ Email_____ ☐

Please indicate if you would prefer to complete the questionnaires
by post ☐ online ☐

Appendix 19: Food Preference Questionnaire

Please indicate how much you like each food by ticking in the appropriate box
IF YOU HAVE NEVER TRIED A FOOD, TICK THE 1ST BOX ONLY

	Never tried it	I hate it	I don't like it	It's OK	I quite like it	I love it
Beef	□	□	□	□	□	□
Beefburger, hamburger	□	□	□	□	□	□
Lamb	□	□	□	□	□	□
Pork	□	□	□	□	□	□
Chicken	□	□	□	□	□	□
Turkey	□	□	□	□	□	□
Bacon	□	□	□	□	□	□
Ham	□	□	□	□	□	□
Sausages	□	□	□	□	□	□
Liver	□	□	□	□	□	□
Pate	□	□	□	□	□	□
Liver sausage	□	□	□	□	□	□
Fish: fried in batter or breadcrumbs	□	□	□	□	□	□
Mackerel	□	□	□	□	□	□
Tuna	□	□	□	□	□	□

	Never tried it	I hate it	I don't like it	It's OK	I quite like it	I love it
Baked beans	◻	◻	◻	◻	◻	◻
Lentils, chickpeas etc	◻	◻	◻	◻	◻	◻
Tofu	◻	◻	◻	◻	◻	◻
Quorn	◻	◻	◻	◻	◻	◻
Soya meat	◻	◻	◻	◻	◻	◻
TVP (textured vegetable protein)	◻	◻	◻	◻	◻	◻
Vegeburger, vegesausage	◻	◻	◻	◻	◻	◻
Nuts, eg peanuts, nut dishes	◻	◻	◻	◻	◻	◻
Eggs: boiled, poached	◻	◻	◻	◻	◻	◻
Eggs: scrambled	◻	◻	◻	◻	◻	◻
Eggs: fried	◻	◻	◻	◻	◻	◻
Moussaka	◻	◻	◻	◻	◻	◻
Meat pies	◻	◻	◻	◻	◻	◻
Pork pies	◻	◻	◻	◻	◻	◻
Pizza	◻	◻	◻	◻	◻	◻
Sausage rolls	◻	◻	◻	◻	◻	◻
Shepherd's pie	◻	◻	◻	◻	◻	◻
Bread, rolls	◻	◻	◻	◻	◻	◻
Cream crackers, cheese biscuits	◻	◻	◻	◻	◻	◻
Ryvita	◻	◻	◻	◻	◻	◻

	Never tried it	I hate it	I don't like it	It's OK	I quite like it	I love it
Cheese (processed) eg Dairylea, Kraft	◻	◻	◻	◻	◻	◻
Cheese (hard), eg cheddar	◻	◻	◻	◻	◻	◻
Cheese (low fat), eg cottage cheese	◻	◻	◻	◻	◻	◻
Cheese (soft): eg Brie, camembert	◻	◻	◻	◻	◻	◻
Bran cereals: e.g. All Bran	◻	◻	◻	◻	◻	◻
Muesli: e.g. Alpen	◻	◻	◻	◻	◻	◻
Porridge or Ready Brek	◻	◻	◻	◻	◻	◻
Rice or corn cereal, eg Cornflakes, Rice Krispies	◻	◻	◻	◻	◻	◻
Sugared cereal, eg Ricicles, Frosties, Sugar Puffs	◻	◻	◻	◻	◻	◻
Potatoes: boiled, mashed or jacket	◻	◻	◻	◻	◻	◻
Potatoes: chips	◻	◻	◻	◻	◻	◻
Potatoes: roast, fried	◻	◻	◻	◻	◻	◻

	Never tried it	I hate it	I don't like it	It's OK	I quite like it	I love it
Broccoli	◻	◻	◻	◻	◻	◻
Cabbage, spring greens	◻	◻	◻	◻	◻	◻
Carrots	◻	◻	◻	◻	◻	◻
Green beans	◻	◻	◻	◻	◻	◻
Leeks	◻	◻	◻	◻	◻	◻
Marrow, courgettes	◻	◻	◻	◻	◻	◻
Mushrooms	◻	◻	◻	◻	◻	◻
Onions	◻	◻	◻	◻	◻	◻
Parsnips	◻	◻	◻	◻	◻	◻
Peas	◻	◻	◻	◻	◻	◻
Salad greens, eg lettuce	◻	◻	◻	◻	◻	◻
Tomatoes	◻	◻	◻	◻	◻	◻
Sprouts	◻	◻	◻	◻	◻	◻
Turnips	◻	◻	◻	◻	◻	◻
Swedes	◻	◻	◻	◻	◻	◻
Celery	◻	◻	◻	◻	◻	◻
Yams	◻	◻	◻	◻	◻	◻
Plantain	◻	◻	◻	◻	◻	◻

	Never tried it	I hate it	I don't like it	It's OK	I quite like it	I love it
Apples	◻	◻	◻	◻	◻	◻
Bananas	◻	◻	◻	◻	◻	◻
Oranges	◻	◻	◻	◻	◻	◻
Tangerines, satsumas, clementines	◻	◻	◻	◻	◻	◻
Grapes	◻	◻	◻	◻	◻	◻
Peaches, nectarines	◻	◻	◻	◻	◻	◻
Pears	◻	◻	◻	◻	◻	◻
Plums	◻	◻	◻	◻	◻	◻
Strawberries	◻	◻	◻	◻	◻	◻
Raspberries	◻	◻	◻	◻	◻	◻
Avocado pears	◻	◻	◻	◻	◻	◻
Milk (skimmed)	◻	◻	◻	◻	◻	◻
Milk (semi-skimmed)	◻	◻	◻	◻	◻	◻
Milk (full fat)	◻	◻	◻	◻	◻	◻
Fizzy drinks, eg Coca Cola, Fanta	◻	◻	◻	◻	◻	◻
Fruit juice: 100% pure	◻	◻	◻	◻	◻	◻
Butter	◻	◻	◻	◻	◻	◻
Margarine	◻	◻	◻	◻	◻	◻
Cream	◻	◻	◻	◻	◻	◻
Biscuits: chocolate	◻	◻	◻	◻	◻	◻
Cakes	◻	◻	◻	◻	◻	◻
Buns/pastries, eg scones, Danish pastries	◻	◻	◻	◻	◻	◻

	Never tried it	I hate it	I don't like it	It's OK	I quite like it	I love it
Fruit pie/tarts/crumbles	◻	◻	◻	◻	◻	◻
Sponge pudding	◻	◻	◻	◻	◻	◻
Ice cream	◻	◻	◻	◻	◻	◻
Ice lollies	◻	◻	◻	◻	◻	◻
Custard	◻	◻	◻	◻	◻	◻
Blancmange	◻	◻	◻	◻	◻	◻
Dairy desserts, eg mousse	◻	◻	◻	◻	◻	◻
Yogurt, fromage frais: eg Muller, Petits Filous	◻	◻	◻	◻	◻	◻
Crisps	◻	◻	◻	◻	◻	◻
Savoury snacks, eg Twiglets, Cheddars	◻	◻	◻	◻	◻	◻
Chocolate	◻	◻	◻	◻	◻	◻
Sweets: fruit	◻	◻	◻	◻	◻	◻
Sweets: mints	◻	◻	◻	◻	◻	◻
Sweets: toffee, fudge	◻	◻	◻	◻	◻	◻
Sauces: salad dressing, mayonnaise	◻	◻	◻	◻	◻	◻
Sauces: warm, savoury eg gravy	◻	◻	◻	◻	◻	◻
Rice	◻	◻	◻	◻	◻	◻
Pasta	◻	◻	◻	◻	◻	◻

Appendix 20: Infant feeding and allergy history questionnaire (school-aged children study)

Please complete this form by ticking the appropriate boxes. Please answer every question.

Please send back to Kate Maslin in the enclosed pre paid envelope. If you have any queries, please phone Kate Maslin, Allergy Dietitian on 01983 534373 or email: kate.maslin@port.ac.uk

Study ID number		Child's Date of Birth		Today's Date	

Please indicate who is completing the questionnaire:

Researcher ☐ Mother ☐ Father □ Grandparent ☐ Guardian ☐

Other □_____

Part 1 : General information about your child and your family

1.1 Your child's gender: Female ☐ Male ☐

1.2 What is your child's ethnic background? *Please tick only one option.*

White British ☐ White European ☐ White Other ☐

Black: British ☐ Black Caribbean ☐ Black African ☐

Black Other ☐ Asian: Indian ☐ Asian: Pakistani ☐

Asian: Bangladeshi ☐ Asian: Other ☐ Mixed Race ☐

Chinese ☐ Other ☐

1.3 What is the child's parents' occupational status?
Please tick only one for each parent.

Mother		**Father**	
Student	☐	Student	☐
Self-employed	☐	Self-employed	☐
Full-time employed	☐	Full-time employed	☐
Part-time employed	☐	Part-time employed	☐
Retired	☐	Retired	☐
Unemployed	☐	Unemployed	☐
Other:	☐	Other:	☐

1.4 What is the highest qualification the child's mother and father hold?
Please tick only one for each parent.

Mother		**Father**	
None	☐	None	☐
GCSE (or equivalent qualification)	☐	GCSE (or equivalent qualification)	☐
A-level (or equivalent qualification)	☐	A-level (or equivalent qualification)	☐
Graduate level qualification	☐	Graduate level qualification	☐
Post-graduate qualification	☐	Post-graduate qualification	☐

Part 2. Family History of Allergy

2.1 Has any of the following persons ever had asthma?

MOTHER		**FATHER**		**ANY SIBLING OF CHILD**	
YES	☐	YES	☐	YES	☐
NO	☐	NO	☐	NO	☐
DON'T KNOW	☐	DON'T KNOW	☐	DON'T KNOW	☐
				NOT APPLICABLE	☐

2.2 Has any of the following persons ever had hayfever?

MOTHER		**FATHER**		**ANY SIBLING OF CHILD**	
YES	☐	YES	☐	YES	☐
NO	☐	NO	☐	NO	☐
DON'T KNOW	☐	DON'T KNOW	☐	DON'T KNOW	☐
				NOT APPLICABLE	☐

2.3 Has any of the following persons ever had an itchy rash which was coming and going for at least six months?

MOTHER		**FATHER**		**ANY SIBLING OF CHILD**	
YES	☐	YES	☐	YES	☐
NO	☐	NO	☐	NO	☐
DON'T KNOW	☐	DON'T KNOW	☐	DON'T KNOW	☐
				NOT APPLICABLE	☐

2.4 Has any of the following persons ever had wheezing or whistling in the chest at any time in the past?

MOTHER		**FATHER**		**ANY SIBLING OF CHILD**	
YES	☐	YES	☐	YES	☐
NO	☐	NO	☐	NO	☐
DON'T KNOW	☐	DON'T KNOW	☐	DON'T KNOW	☐
				NOT APPLICABLE	☐

2.5 Has any of the following persons ever suffered from an itchy, stuffy or runny nose and/or swollen, itchy eyes when they did not have a cold?

MOTHER		FATHER		ANY SIBLING OF CHILD	
YES	☐	YES	☐	YES	☐
NO	☐	NO	☐	NO	☐
DON'T KNOW	☐	DON'T KNOW	☐	DON'T KNOW	☐
				NOT APPLICABLE	☐

2.6 Has the mother of the child ever suffered from symptoms of food allergy or intolerance?

YES ☐ NO ☐ DON'T KNOW ☐

2.7 Has the father of the child ever suffered from symptoms of food allergy or intolerance?

YES ☐ NO ☐ DON'T KNOW ☐

2.9 Has any sibling of the child ever suffered from symptoms of food allergy or intolerance?

YES ☐ NO ☐ DON'T KNOW ☐

NOT APPLICABLE ☐

Part 3: Questions about Child's Allergy History

3.1 Has the child ever had wheezing or whistling in the chest ?

YES ☐ NO ☐ DON'T KNOW ☐

3.2 Has the child had a dry cough at night, apart from the cough associated with a cold or a chest infection?

YES ☐ NO ☐ DON'T KNOW ☐

3.3 Has the child ever had an itchy rash that was coming and going ?

YES ☐ NO ☐ DON'T KNOW ☐

3.31 Did you identify a cause for this itchy rash ?

YES ☐ Please state

_____

NO ☐ NOT APPLICABLE ☐

3.4 Has the child ever suffered from vomiting (> 1 tablespoon)?

YES ☐ NO ☐ DON'T KNOW ☐

3.4.1 Did you identify a cause for this vomiting ?

YES ☐ Please state _____

NO ☐ NOT APPLICABLE ☐

3.5 Has the child ever suffered from diarrhoea ?

YES ☐ NO ☐ DON'T KNOW ☐

3.5.1 Did you identify a cause for this diarrhoea ?

YES ☐ Please state _____

NO ☐ NOT APPLICABLE ☐

3.6 Has the child ever suffered from constipation ?

YES ☐ NO ☐ DON'T KNOW ☐

3.6.1 Did you identify a cause for this constipation ?

YES ☐ Please state _____

NO ☐ NOT APPLICABLE ☐

3.7 Has the child ever suffered from abdominal distension (bloating) ?

YES ☐ NO ☐ DON'T KNOW ☐

3.7.1 Did you identify a cause for this abdominal distension (bloating) ?

YES ☐ Please state _____

NO ☐ NOT APPLICABLE ☐

3.8 Has the child ever suffered from colic/tummy ache ?

YES ☐ NO ☐ DON'T KNOW ☐

3.8.1 Did you identify a cause for this colic/tummy ache ?

YES ☐ Please state _____

NO ☐ NOT APPLICABLE ☐

3.9 Has the child ever suffered from any other food related problems ?

YES ☐ Please state problem_____

NO ☐ DON'T KNOW ☐

3.9.1 Did you identify a cause for this food related problem ?

YES ☐ Please state _____

NO ☐ NOT APPLICABLE ☐

Part 4: Dietary exclusion

4.1 Has the child ever had food excluded from their diet due to food allergy?

YES ☐ NO ☐ *If no, please go to part 5*

4.2 Which food(s) were excluded?

Milk and milk products ☐ Egg ☐ Wheat ☐ Soya ☐

Fish ☐ Peanut ☐ Tree nuts ☐

Other, please state _____

4.3 At what age were the foods excluded (approximately)?

Food	**Age excluded**

4.4 How was the food allergy diagnosed?

Skin prick test ☐ Blood test ☐ Exclusion diet ☐

Not formally diagnosed☐ Don't know ☐ Food challenge ☐

Other method please state _____

4.5 What age was the child when these foods were successfully reintroduced into their diet?

Food	**Age reintroduced**

Part 5 Child's growth

5.1 Was the child born at full term?

Yes ☐ No ☐ please state gestation in weeks_____

5.2 What was the child's birthweight?

Please state_____ Don't know ☐

5.3 Most recent weight _____ **Date**_____

5.4 Most recent height _____ **Date** _____

5.5 Waist circumference_____

Part 6: Feeding history

6.1 Was the child breastfed at all Yes ☐ No ☐

IF yes, for how long? *Please tick one box*

Up to 1 month ☐ Up to 2 months ☐ Up to 3 months ☐

Up to 4 months ☐ Up to 5 months ☐ Up to 6 months ☐

Up to 9 months ☐ Up to 12 months ☐ 12 months or more ☐

Don't know ☐

6.2 Was the child given formula milk at any point ?

(either as a top up OR as the baby's main drink) Yes ☐ No ☐

IF yes, when were they first given formula milk? *Please tick one box*

Before 1 month ☐ Between 1-2 months ☐ Between 2-3months ☐

Between 3-4 months ☐ Between 4-5 months ☐ Between 5-6 months ☐

Between 6-9 months ☐ Between 9-12 months ☐ After 12 months ☐

Don't know ☐

6.3 If the child was given formula milk, which ones?

Name of formula milk	How long for approximately

6.4 At what age was the child first given solid food? _____weeks old

If not sure, please could you estimate age in weeks_____

6.5 At what age was the child first given lumpy food?_____weeks old

If not sure, please could you estimate age in weeks_____

6.6 At what age was the child first given finger foods? _____weeks old
If not sure, please could you estimate age in weeks_____
6.7 What were the first foods that were introduced to the child?

Food 1_____

Food 2_____

Food 3_____

Food 4_____

6.8 Was the child predominantly weaned onto homemade foods or pre prepared baby food ? Homemade ☐ Pre prepared ☐ Mixture of both ☐

6.9 Was the child a fussy eater at the following ages?

6-12 months	Yes	☐	No	☐	Don't Know☐
1-2 years old	Yes	☐	No	☐	Don't Know☐
2-3 years old	Yes	☐	No	☐	Don't Know☐

Part 7: Questions about the child's current diet
7.1 What type of diet does the child eat?

Normal ☐ Vegetarian ☐ Vegan ☐ Other ☐ please state_____

7.2 Does the child currently have any medical conditions that affect their diet (e.g. diabetes)?

Yes ☐ please state _____No ☐

7.3 Does the child currently take any dietary supplements (e.g. vitamins)?

Yes ☐ please state _____No ☐

7.4 Has the child ever been seen by a dietitian?

Yes ☐ please state how many times they have been seen_____No ☐

7.5 If yes, for what reason?

Food allergy ☐ Other reason, ☐ please state _____

7.6 How much attention do you pay to the child's diet in terms of healthy eating?
Please tick one box.

Very little ☐ Somewhat ☐ A great deal ☐

Appendix 21: Food diary

Study number_____

How to fill in your diary

It is very important that you **do not change what you normally eat or drink** just because you are keeping a diary. Try to write down what you are eating or drinking as soon as you can and not leave it until the end of the day. Record food and drink eaten at home **and away from home**, such as at school or at a friend's house.

Describe your food and drink giving **as much detail** as you can. **Include any extras** like sugar and milk in your tea or cereal, **butter or other spreads on your bread and sauces such as ketchup** and mayonnaise.

If you know **how the food was cooked** (eg. roast, baked, boiled, fried), please record this. If you're unsure about how the food was cooked, please ask the person who prepared the food if possible.

If you have eaten any **homemade dishes** eg. a casserolle, please make sure the ingredients and cooking method are recorded in the space provided. You may need to ask the person who prepared the dish to help you with this. If you have eaten **any take-aways** or any made up dishes not prepared at home such as at a friend's house or in a restaurant, please record as much detail as you can about what was in the dish eg. Vegetable curry containing chickpeas, aubergine, onion and tomato.

Brand: Please make a note of the brand name (eg. Heinz, Walkers, Hovis) if you know it.

Amount eaten: You can write S (small), M (medium) or L (large) portion, or specify size of packet (eg. 25g Crisps, 120g Yogurt), or number of individual items (eg. biscuits), or slices (eg. cake, pizza, ham), or teaspoons (eg. sugar), or tablespoons (ketchup, peas).

For drinks you can write glass (tell us the **size of the glass** or volume using page 18 as a guide), cup or mug. You can also write the weight or **volume from the labels on the packaging** (e.g. 330ml can of coke).

Food labels/wrappers: Please keep the labels or packaging from snacks, sweets, bought sandwiches and ready meals and put them in the envelope provided.

Food Diary

Please complete four days. Please refer to instructions for advice about how to fill in the food diary.

DAY 1 **DAY of week :** **DATE:**

Time	What food	Brand Name	Amount Eaten
6am-9am			
9am – 12 noon			
12 noon – 2pm			
2pm-5pm			
5pm-8pm			
8pm-10pm			
10pm – 6am			

DAY 1 QUESTIONS

Was the amount of food that you had today about what you usually have, less than usual or more than usual?

Usual amount ☐ Less than usual ☐ More than usual ☐

If more or less than usual, please tell us why?

_____

_____

_____

Was the amount of drinks that you had today about what you usually have, less than usual or more than usual?

Usual amount ☐ Less than usual ☐ More than usual ☐

If more or less than usual, please tell us why?

_____

_____

_____

Did you finish all the food and drink that you recorded in the diary today? Yes ☐ No ☐

Write in recipe or ingredients of home made dishes or take away dishes eaten today

NAME of DISH_____ How many people does this serve?_____

Ingredients	Amount	Ingredients	Amount

DAY 2 **DAY of week :** **DATE:**

Time	What food	Brand Name	Amount Eaten
6am-9am			
9am – 12 noon			
12 noon – 2pm			
2pm-5pm			
5pm-8pm			
8pm-10pm			
10pm – 6am			

DAY 2 QUESTIONS

Was the amount of food that you had today about what you usually have, less than usual or more than usual?

Usual amount ☐ Less than usual ☐ More than usual ☐

If more or less than usual, please tell us why?

_____

_____

_____

Was the amount of drinks that you had today about what you usually have, less than usual or more than usual?

Usual amount ☐ Less than usual ☐ More than usual ☐

If more or less than usual, please tell us why?

_____

_____

_____

Did you finish all the food and drink that you recorded in the diary today? Yes ☐ No ☐

Write in recipe or ingredients of home made dishes or take away dishes eaten today

NAME of DISH_____ How many people does this serve?_____

Ingredients	Amount	Ingredients	Amount

DAY 3 **DAY of week :** **DATE:**

Time	What food	Brand Name	Amount Eaten
6am-9am			
9am – 12 noon			
12 noon – 2pm			
2pm-5pm			
5pm-8pm			
8pm-10pm			
10pm – 6am			

DAY 3 QUESTIONS

Was the amount of food that you had today about what you usually have, less than usual or more than usual?

Usual amount ☐ Less than usual ☐ More than usual ☐

If more or less than usual, please tell us why?

_____

_____

_____

Was the amount of drinks that you had today about what you usually have, less than usual or more than usual?

Usual amount ☐ Less than usual ☐ More than usual ☐

If more or less than usual, please tell us why?

_____

_____

_____

Did you finish all the food and drink that you recorded in the diary today? Yes ☐ No ☐

Write in recipe or ingredients of home made dishes or take away dishes eaten today

NAME of DISH_____ How many people does this serve?_____

Ingredients	Amount	Ingredients	Amount

DAY 4 **DAY of week :** **DATE:**

Time	What food	Brand Name	Amount Eaten
6am-9am			
9am – 12 noon			
12 noon – 2pm			
2pm-5pm			
5pm-8pm			
8pm-10pm			
10pm – 6am			

DAY 4 QUESTIONS

Was the amount of food that you had today about what you usually have, less than usual or more than usual?

Usual amount ☐ Less than usual ☐ More than usual ☐

If more or less than usual, please tell us why?

_____

_____

_____

Was the amount of drinks that you had today about what you usually have, less than usual or more than usual?

Usual amount ☐ Less than usual ☐ More than usual ☐

If more or less than usual, please tell us why?

_____

_____

_____

Did you finish all the food and drink that you recorded in the diary today? Yes ☐ No ☐

Write in recipe or ingredients of home made dishes or take away dishes eaten today

NAME of DISH_____ How many people does this serve?_____

Ingredients	Amount	Ingredients	Amount

Please post back the food diary in the prepaid envelope or email to kate.maslin@port.ac.uk.Please email or phone Kate on 01983 534383 if you have any questions about the food diary. **Thank you**

Appendix 22: Taste preference test

Taste Game

Drink 1:_____	
Super good	☐
Really good	☐
Good	☐
Just a little good	☐
Maybe good or maybe bad	☐
Just a little bad	☐
Bad	☐
Really bad	☐
Super bad	☐

Drink 2:_____	
Super good	☐
Really good	☐
Good	☐
Just a little good	☐
Maybe good or maybe bad	☐
Just a little bad	☐
Bad	☐
Really bad	☐
Super bad	☐

Appendix 23: University Hospital Southampton research and development approval letter

University Hospital Southampton NHS Foundation Trust

Please reply to: Research and Development
90th - Level L, Laboratory & Pathology
Block SCBR - MP 136
Southampton University Hospitals NHS

Telephone: 023 8120 5070
Fax: 023 8120 8078
E-mail: sharon.atwill@uhs.nhs.uk

Professor Graham Roberts
Child Health Directorate
Level F, Mailpoint 803
Southampton General Hospital
Tremona Road
Southampton
SO16 6YD

29 July 2014

Dear Professor Roberts

ID: RHM CHI0667 **The effect of cow's milk exclusion diet substitute formula in infancy on childhood eating habits**

EudraCT:

Thank you for submitting all the required documentation for Trust R&D approval. I write to inform you that your study has full UHS R&D approval. Please find attached the Conditions of Trust R&D approval which you are obliged to adhere to.
Please note that according to the 70 day benchmark you should aim to recruit your first patient by 26 September 2014.
You are required to keep copies of all your essential documents relating to this study. Please download a copy of the relevant Investigator Site File template from the R&D website:
http://www.uhs.nhs.uk/Research/For-investigators/Sitefile.aspx
Your project is subject to R&D monitoring and you will be contacted by our office to arrange this.
Please note: A condition of approval is that any changes need to be timeously notified to the R&D office. This includes providing copies of:
. All NRES substantial amendments and favourable opinions;
. All Serious Adverse Events (SAEs);
. NRES Annual Progress Reports;
. Annual MHRA Safety Reports;
. NRES End of Study Declaration;
. Notifications of significant breaches of GCP or protocol
Please quote the above RHM No. On any correspondence with our office.
Should you, or any of your team, require training in any of the policies and procedures required to ensure compliance with the conditions of approval, please refer to the R&D Training website http://www.uhs.nhs.uk/Research/For-investigators/Mandatory-training-governance-and-safety-management/Mandatory-training-governance-and-safety-management.aspx for an up-to-date calendar of training events.

Yours sincerely

Sharon Atwill

Research Governance Officer

Appendix 24: Parent information sheet (school-aged children study)

The David Hide Asthma & Allergy Research Centre
St. Mary's Hospital
Newport
Isle of Wight
PO30 5TP

Direct Tel. No. (01983) 534178
Email: kate.maslin@port.ac.uk

Eating habits in children with and without previous food allergy

Information Sheet for Parent/Guardian

Dear Parent(s)/Guardian(s)
We would like to thank you for taking the time to read this study information sheet. We would also like to thank you and your child again for previously helping us with the FAIR/PIFA study. I would now like to invite you to help us with this study of children's eating habits. Before you make any decision about involvement in the study it is important for you to understand why the research is being done and what it will involve.

Please read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear, or if you would like more information, by contacting us on the numbers provided. Take time to decide whether or not you wish to take part.

What is the purpose of the study?
The purpose of this study is to assess food preferences and eating habits in children. We know from previous research that the type of food older children like to eat can be influenced by the type of milk and food they were fed as a baby. Therefore we would like to compare a group of children who ate a normal diet in infancy, with a group of children who ate a special diet for food allergy in infancy.

Why has my child been chosen?
The study involves a subgroup of children who previously took part in the FAIR study. We have selected all the children identified as having eaten a special diet for food allergy in infancy. We have also randomly selected a group of children who followed a normal diet in infancy as a comparison group.

Does my child have to take part?
It is up to you to decide whether or not to take part. If you decide to take part we suggest that you keep this information sheet. Please could you return both consent forms (adult consent form and child's assent form) indicating your willingness to take part in the prepaid envelope provided. You will receive a copy of the signed consent forms when attending the appointment. You and your child are still free to withdraw at any time and without giving a reason. Your child will under no circumstances be asked to do anything against their wishes. A decision to

withdraw at any time, or a decision not to take part, will not affect any medical care your child may receive in future.

What will happen if we decide to take part?
If you decide to take part in the study we would ask you and your child:

Part (i) To attend the Asthma and Allergy Centre/Southampton General Hospital for an appointment, which will last approximately 1 hour.
As part of this appointment:
a. We will ask you some questions about your child's current eating habits and their feeding history as a baby.
b. We will ask your child to complete a simple questionnaire about their food likes and dislikes.
c. We will ask your child to complete a taste preference game. The game will involve tasting a small amount (about one tablespoon) of some different flavoured waters and telling us if they like them or not. There will be five flavoured waters (sweet, sour, salty, bitter and savoury). Your child will be allowed to sip plain water in between tasting the flavoured waters.
d. We will measure your child's weight, height and around their waist. Your child will be asked to remove their shoes and coat for the measurements. Measurements will take place in private, with only you, your child and the dietitian present.

Part (ii) To complete a four day food diary at home, where we would like you to write down everything your child eats and drinks for four days. The dietitian will explain how to complete the food diary and answer any questions you may have.

We would like you and your child to participate in all parts of the study. However if it is not possible for you to attend the allergy centre or you choose not to, we can arrange for you to complete the questionnaires online or by post.

What are the benefits of taking part?
Most children participating in the study will be healthy volunteers. Some of the children may have asthma, eczema and other allergies. The study does not include any treatment for any condition. However, your GP will be informed of your child's participation if any medical treatment is required or any nutritional issues highlighted.

What are the possible disadvantages and risks of taking part?
There are no disadvantages or risks in taking part in this study. The only issue is the inconvenience of completing a questionnaire and attending the allergy centre for an appointment.

What if there is a problem?
If you have any questions or concerns, please contact Kate Maslin, Allergy Dietitian, at The David Hide Asthma and Allergy Research Centre, St Mary's Hospital, Newport, Isle of Wight. Telephone: 01983 534373. Email: kate.maslin@port.ac.uk. If you still have questions or concerns, you can contact Dr. Carina Venter, The David Hide Asthma and Allergy Research Centre, St Mary's Hospital, Newport, Isle of Wight. Telephone: 01983 534373. Email:carina.venter@port.ac.uk. or Alexandra Punter (Lead for Research and Development, St Mary's Hospital, Newport, Isle of Wight; email alexandra.punter@iow.nhs.uk).
In the highly unlikely event that something does go wrong and you are harmed during the research study there are no special compensation arrangements. If you are harmed and this is due to someone's negligence then you may have grounds for a legal action for compensation

against St Mary's Hospital but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you.

Will my child get paid for his/her participation?

We will provide a small gift token to your child as a thank you for taking part. We will reimburse any travel expenses incurred by taking part.

What is the duration of the study?

The study will involve your visit to the allergy centre and completion of the food diary for four days after your appointment. Once you have returned the food diary to us, we may phone you to clarify some details. Nothing else will be required of you.

Will my child's taking part in the study be kept confidential?

Yes. All the information about your child's participation in this study will be kept confidential. Only the study personnel will have access to your child's personal details. Your child will not be individually identified in any reports or publications resulting from the study. We will keep your child's data on file for use in future studies approved by the Research Ethics Committee.

What will happen to the results of the research study?

We aim to publish the results of the study in medical journals so that other doctors and researchers can use our data and provide better care for their patients.

Who is organising and funding the research?

The researchers at The David Hide Asthma and Allergy Research Centre and the University of Portsmouth are organising and carrying out this study. The study is being supported by the National Institute of Health Research (NIHR) and Danone Baby Nutrition.

Who has reviewed the study?

The study has been reviewed by the Berkshire Research Ethics Committee on 9th May 2013 and was given a favourable ethical opinion for conduct in the NHS.

How long do I have to decide whether my child should take part?

Your decision to participate in this study is entirely voluntary. You should take as much time as you need, but we do ask you to let us know of your participation by *insert appropriate date*.

What do I do now?

If you are interested in joining the study please sign the enclosed two consent forms (parent's and child's). Please then return these to me in the enclosed envelope. I will contact you to arrange a convenient appointment time for you to attend the allergy centre. To help me do this and if you are happy to, could you please also write your phone number and email address on the consent form.

If you would prefer to complete the questionnaires online or by post, please indicate this on the consent form and they can be posted or emailed to you. If you have any questions at all then please do not hesitate to contact me and I will be happy to speak to you.

Thank you for your time

Kate Maslin,
Allergy Dietitian, David Hide Asthma & Allergy Centre & PhD Student, University of Portsmouth.
Carina Venter
Senior Allergy Dietitian, David Hide Asthma & Allergy Centre

Appendix 25: Child information sheet

The David Hide Asthma & Allergy Research Centre
St. Mary's Hospital
Newport
Isle of Wight
PO30 5TG

Direct Tel: (01983) 534178
Direct Fax: (01983) 534907

Eating habits in children with and without previous food allergy

Child Information sheet

What is this study about?

Some children are fussy eaters. Other children like lots of different types of food. We would like to find out why children your age like different foods. Some children had special milk and food when they were a baby because of a food allergy. Most babies don't have food allergies. We would like to find out if the milk and food you had when you were a baby affects the foods you like now.

DO I have to take part?

No, it is entirely up to you and your parents if you want to.

What will happen to me if I take part?

We will ask you to come to the allergy centre for about an hour with your mum or dad. We will ask you and your mum or dad some questions about the foods you like to eat. We will ask you to do some taste games. We will measure your weight and height. We will measure around your tummy with a measuring tape. When you go home, we will ask you and your mum and dad to write down everything you eat and drink for four days.

What will I have to do for the taste games?

We would like you to taste a small amount of different flavoured waters and tell us whether you like them or not. You will be allowed to drink some plain water after the flavoured waters. The taste games will take about 10 minutes.

Might anything about the research upset me?

If there is anything you do not like or do not want to do – just say so. You do not have to taste anything you do not want to.

Will anyone else know I'm doing this?

No – we will not tell anyone that you are involved, unless you want us to.

What happens when the research stops?

The researcher, Kate, will be able to tell doctors, nurses and dietitians about what foods children your age like and eat.

Did anyone else check the study is OK to do?

Before any research is allowed to happen, it has to be checked by a group of people called a Research Ethics Committee. They make sure that the research is fair. This project has been checked by the Berkshire Research Ethics Committee.

What if I don't want to do the study anymore?

If at any time you don't want to do the study any more, just tell your parents, or Kate, the researcher. They will not be cross with you.

Thank you for reading this – please talk it through with your parents. You are welcome to phone us if you think of any questions- ask for Kate on 01983 534178.

Please discuss with your parents and if interested let us know

Appendix 26: Parent consent form (school-aged children study)

The David Hide Asthma & Allergy Research Centre
St. Mary's Hospital
Newport
Isle of Wight
PO30 5TG

Direct Tel: (01983) 534178
Email: kate.maslin@port.ac.uk

Eating habits in children with and without previous food allergy

Name of Researcher: Kate Maslin

Parent/Guardian Consent Form

Please initial box

I confirm that I have read and understand the information sheet dated 1st May 2013 (version 1.1) for the above study.	☐
I have now had the opportunity to consider the information, regarding the study, ask questions and have had these answered satisfactorily.	☐
I understand that my child's participation is voluntary and that my child is free to withdraw at any time without giving any reason, without her/his medical care or legal rights being affected.	☐
I understand that relevant sections of my child's medical notes and data collected during the study, may be looked at by individuals from the Isle of Wight NHS Primary Care Trust or from regulatory authorities where it is relevant to my child's taking part in this research	☐
I give permission for these individuals to have access to his/her records.	☐
I understand that my GP will only be informed of my child's participation in the study if my child requires medical treatment or any nutritional concerns are highlighted.	☐
I consent to my child undergoing taste preference tests.	☐
I consent to my child having their weight, height and waist circumference measured.	☐

_____ _____ _____

Parent/Guardian Name (in block letters) Signature Date

_____

Child's Name

_____ _____ _____

Researcher Signature Date

I am happy for you to contact me by:

Phone_____ ☐ Email_____ ☐

Please indicate if you would prefer to complete the questionnaires by post ☐

online ☐

Appendix 27: Child consent form

The David Hide Asthma & Allergy Research Centre
St. Mary's Hospital
Newport
Isle of Wight
PO30 5TG

Direct Tel: (01983) 534178
Email: kate.maslin@port.ac.uk

Children's Consent Form

Name of Researcher: Kate Maslin

Please circle the answers you agree with or ask your parents to help you.

Do you understand what we would like you to do for the study?	Yes / No
Have you asked all the questions you want to about the research?	Yes / No
Did you understand all the answers you got?	Yes / No
Do you understand it's OK to stop taking part at any time?	Yes / No
Are you happy to answer questions about the foods you eat?	Yes / No
Are you happy to do the taste game? (You can stop the taste game at any stage)	Yes / No
Are you happy to have your weight, height and waist measured?	Yes / No
Are you happy to help your parents fill in the food diary at home?	Yes / No

If you do want to take part, you can write your name below or ask your parents to help you to do so.

Your name _____ Date _____

Researcher Name _____ Sign _____ Date _____

Appendix 28: Recruitment letter for children recruited from NHS clinic records (school-aged children study)

The David Hide Asthma & Allergy Research Centre
St. Mary's Hospital
Newport
Isle of Wight
PO30 5TP

Direct Tel. No. (01983) 534373
Email: kate.maslin@port.ac.uk

Eating habits in children with and without previous food allergy

Information Sheet for Parent/Guardian

Dear Parent(s)/Guardian(s)
My name is Kate Maslin and I am an allergy dietitan at the David Hide Asthma and Allergy Centre. I am conducting a research project on behalf of the University of Portsmouth. I would like to thank you for taking the time to read this study information sheet. I would now like to invite you to help us with this study of children's eating habits. Before you make any decision about involvement in the study it is important for you to understand why the research is being done and what it will involve.

Please read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear, or if you would like more information, by contacting us on the number and email address provided. Take time to decide whether or not you wish to take part.

What is the purpose of the study?
The purpose of this study is to assess food preferences and eating habits in children. We know from previous research that the type of food older children like to eat can be influenced by the type of milk and food they were fed as a baby. Therefore we would like to compare a group of children who ate a normal diet in infancy, with a group of children who ate a special diet for food allergy in infancy.

Why has my child been chosen?
The study involves children aged 7-13 years old. Your child has been identified from our records as having attended the allergy centre in the past.

Does my child have to take part?
It is up to you to decide whether or not to take part. If you decide to take part we suggest that you keep this information sheet. Please could you return both consent forms (adult consent form and child's assent form) indicating your willingness to take part in the prepaid envelope provided. You will receive a copy of the signed consent forms when attending the appointment.

You and your child are still free to withdraw at any time and without giving a reason. Your child will under no circumstances be asked to do anything against their wishes. A decision to withdraw at any time, or a decision not to take part, will not affect any medical care your child may receive in future.

What will happen if we decide to take part?
If you decide to take part in the study we would ask you and your child:

Part (i) To attend the Asthma and Allergy Centre for an appointment, which will last approximately 1 hour.
As part of this appointment:
a. We will ask you some questions about your child's current eating habits and their feeding history as a baby.
b. We will ask your child to complete a simple questionnaire about their food likes and dislikes.
c. We will ask your child to complete a taste preference game. The game will involve tasting a small amount (about one tablespoon) of some different flavoured waters and telling us if they like them or not. There will be five flavoured waters (sweet, sour, salty, bitter and savoury). Your child will be allowed to sip plain water in between tasting the flavoured waters.
d. We will measure your child's weight, height and around their waist. Your child will be asked to remove their shoes and coat for the measurements. Measurements will take place in private, with only you, your child and the dietitian present.

Part (ii) To complete a four day food diary at home, where we would like you to write down everything your child eats and drinks for four days. The dietitian will explain how to complete the food diary and answer any questions you may have.

We would like you and your child to participate in all parts of the study. However if it is not possible for you to attend the allergy centre or you choose not to, we can arrange for you to complete the questionnaires online or by post.

What are the benefits of taking part?
Most children participating in the study will be healthy volunteers. Some of the children may have asthma, eczema and other allergies. The study does not include any treatment for any condition. However, your GP will be informed of your child's participation if any medical treatment is required or any nutritional issues highlighted.

What are the possible disadvantages and risks of taking part?
There are no disadvantages or risks in taking part in this study. The only issue is the inconvenience of completing a questionnaire and attending the allergy centre for an appointment.

What if there is a problem?
If you have any questions or concerns, please contact Kate Maslin, Allergy Dietitian, at The David Hide Asthma and Allergy Research Centre, St Mary's Hospital, Newport, Isle of Wight. Telephone: 01983 534373. Email: kate.maslin@port.ac.uk. If you still have questions or concerns, you can contact Dr. Carina Venter, The David Hide Asthma and Allergy Research Centre, St Mary's Hospital, Newport, Isle of Wight. Telephone: 01983 534373. Email:carina.venter@port.ac.uk. or Alexandra Punter (Lead for Research and Development, St Mary's Hospital, Newport, Isle of Wight; email alexandra.punter@iow.nhs.uk).
In the highly unlikely event that something does go wrong and you are harmed during the research study there are no special compensation arrangements. If you are harmed and this is

due to someone's negligence then you may have grounds for a legal action for compensation against St Mary's Hospital but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you.

Will my child get paid for his/her participation?
We will provide a small gift token to your child as a thank you for taking part. We will reimburse any travel expenses incurred by taking part.

What is the duration of the study?
The study will involve your visit to the allergy centre and completion of the food diary for four days after your appointment. Once you have returned the food diary to us, we may phone you to clarify some details. Nothing else will be required of you.

Will my child's taking part in the study be kept confidential?
Yes. All the information about your child's participation in this study will be kept confidential. Only the study personnel will have access to your child's personal details. Your child will not be individually identified in any reports or publications resulting from the study. We will keep your child's data on file for use in future studies approved by the Research Ethics Committee.

What will happen to the results of the research study?
We aim to publish the results of the study in medical journals so that other doctors and researchers can use our data and provide better care for their patients.

Who is organising and funding the research?
The researchers at The David Hide Asthma and Allergy Research Centre and the University of Portsmouth are organising and carrying out this study. The study is being supported by the National Institute of Health Research (NIHR) and Danone Baby Nutrition.

Who has reviewed the study?
The study has been reviewed by the Berkshire Research Ethics Committee on 9th May 2013 and was given a favourable ethical opinion for conduct in the NHS.

How long do I have to decide whether my child should take part?
Your decision to participate in this study is entirely voluntary. You should take as much time as you need, but we do ask you to let us know of your participation by *insert appropriate date*.

What do I do now?
If you are interested in joining the study please sign the enclosed two consent forms (parent's and child's). Please then return these to me in the enclosed envelope. I will contact you to arrange a convenient appointment time for you to attend the allergy centre. To help me do this and if you are happy to, could you please also write your phone number and email address on the consent form.
If you would prefer to complete the questionnaires online or by post, please indicate this on the consent form and they can be posted or emailed to you. If you have any questions at all then please do not hesitate to contact me and I will be happy to speak to you.
Thank you for your time

Kate Maslin
Allergy Dietitian, David Hide Asthma & Allergy Centre & PhD Student, University of Portsmouth

Appendix 29: UPR16 Form

University of Portsmouth

FORM UPR16

Research Ethics Review Checklist

Please include this completed form as an appendix to **your thesis (se Postgraduate Research Student Handbook for more information**

Postgraduate Research Student		**Student ID:**	**677930**
Candidate Name:	**Kate Maslin**		
Department:	**SHSSW**	**First Supervisor:**	**Prof. Tara Dean**
Start Date:		**October 2012**	

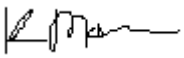
Study Mode and Route:	Part-time ❑ Full-time X❑	

Title of Thesis:	The effect of a cows' milk exclusion diet and substitute formula on childhood eating habits
Thesis Word Count: (excluding ancillary data)	Approx 71, 000

If you are unsure about any of the following, please contact the local representative on your Faculty Ethics Committee for advice. Please note that it is your responsibility to follow the University's Ethics Policy and any relevant University, academic or professional guidelines in the conduct of your study

Although the Ethics Committee may have given your study a favourable opinion, the final responsibility for the ethical conduct of this work lies with the researcher(s).

UKRIO Finished Research Checklist: (If you would like to know more about the checklist, please see your Faculty or Departmental Ethics Committee rep or see the online version of the full checklist at: http://www.ukrio.org/what-we-do/code-of-practice-for-research/)	
a) Have all of your research and findings been reported accurately, honestly and within a reasonable time frame?	**YES**
b) Have all contributions to knowledge been acknowledged?	**YES**
c) Have you complied with all agreements relating to intellectual property, publication and authorship?	**YES**
d) Has your research data been retained in a secure and accessible form and will it remain so for the required duration?	**YES**
e) Does your research comply with all legal, ethical, and contractual requirements?	**YES**

Candidate Statement:	
I have considered the ethical dimensions of the above named research project, and have successfully obtained the necessary ethical approval(s)	
Ethical review number(s) from Faculty Ethics Committee (or from NRES/SCREC):	13/SC/0194 Berkshire NHS Research Ethics Committee
Signed:  ***(Student)***	**Date:** 15th September 2015
If you have *not* submitted your work for ethical review, and/or you have answered 'No' to one or more of questions a) to e), please explain why this is so:	
Signed: ***(Student)***	**Date:**