Timing of complementary feeding:
Its association with growth, diet, iron status, and eating behaviours in infants and toddlers

Antiopi Ntouva

A thesis submitted in partial fulfilment of the requirements of the University of Brighton for the degree of Doctor of Philosophy

March 2014

University of Brighton

To my husband Anthony, my dad Yiorgos, my brother Kostas
and in loving memory of my mum Ioanna (1958-2008)
and my friend Mike Moss (1935-2014)

Abstract

There has been considerable debate over the optimum age of introduction of solid foods in infants and particularly its association with nutrient and iron adequacy for growth and development as well as its impact on eating behaviours, such as feeding difficulties, dietary variety and food preferences. Since infants and toddlers are at a higher risk of developing iron deficiency anaemia, a non-invasive method of measuring haemoglobin could be a useful tool in identifying low haemoglobin levels early and prevent further deterioration of iron status. Since the adoption of the World Health Organisation (WHO) recommendations for exclusive breastfeeding for 6 months, there has been limited data in the UK about how the new guidelines have affected the growth, eating behaviours, dietary intake and iron status. This thesis aimed to address these gaps in the literature in 3 studies: the Brighton's Breast-fed Babies Study, the Haemospect® Validation Study and the GO-CHILD Study. For the Brighton's Breast-fed Babies Study, a 3-day dietary record, anthropometric measurements and a capillary blood sample for measuring haemoglobin levels were obtained from healthy, term, breast-fed infants from Brighton at 8 months of age (n=20) and at 18 months of age (n=19). The association between the age of solids introduction and haemoglobin levels, iron and other nutrient intakes, and reported eating behaviours were examined. Using the same sample from Brighton, a non-invasive method (white-light spectroscopy) was also validated concurrently with the capillary blood sampling technique to check its accuracy in measuring haemoglobin levels at 18 months (The Haemospect® Study). In the GO-CHILD study, a self-complete questionnaire was returned by 230 mothers from Sussex when their infants were aged around 9 months. The age of introduction of solids was considered in relation to the introduction of selected foods, eating behaviours, food preferences and dietary variety respectively. The Brighton's Breast-fed Babies Study provided a unique opportunity to explore the feasibility of conducting a longitudinal study with infants who follow the current feeding recommendations. Even though firm conclusions on the association between the age of solids introduction and growth, diet and iron status could not be drawn from this small sample of infants, useful insights were gained with respect to the recruitment challenges in this area of research and recommendations for the design of future studies were made. In the Haemospect® study, the non-invasive technique was not successful in measuring accurately haemoglobin levels at 18 months. Timing of solids introduction was not found to be an important predictor of food preferences, dietary variety and eating behaviours at 9 months in the GO-CHILD study. It is hoped that the insights gained into the challenges faced during these studies will assist in the successful design of future studies with similar population groups.

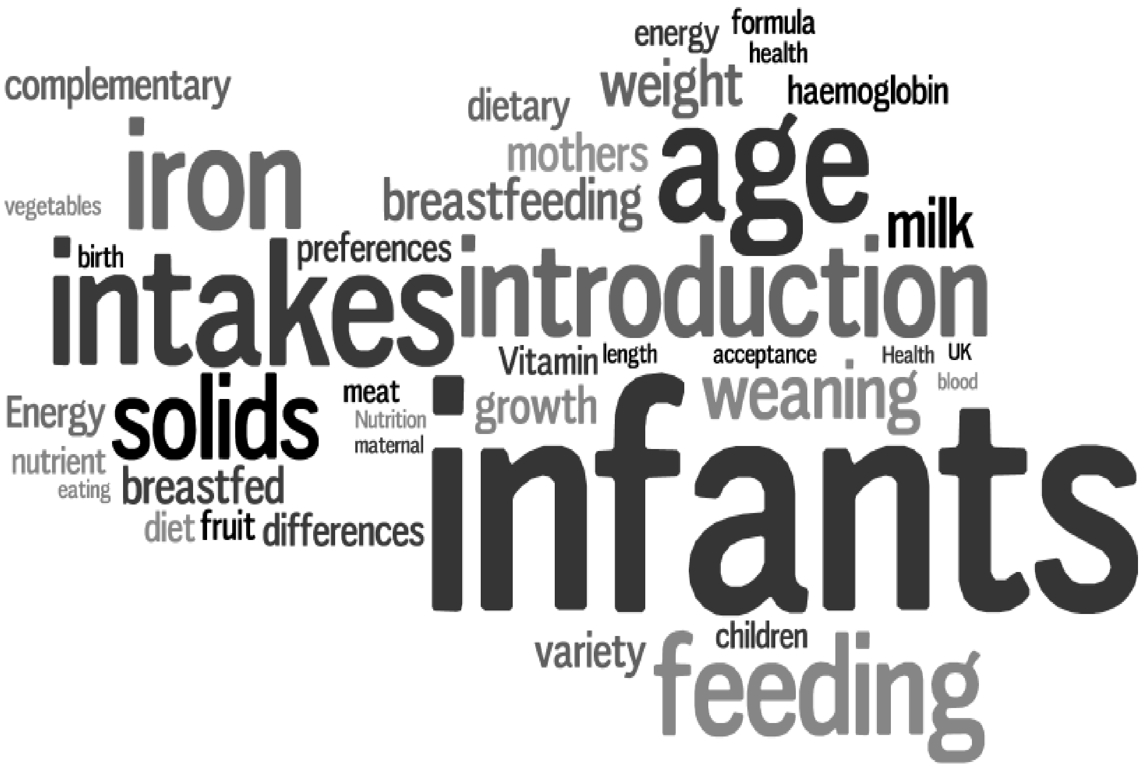

energy formula health
complementary
weight
haemoglobin
dietary
iron
age
mothers
vegetables
breastfeeding
milk
birth
preferences
intakes
introduction
Vitamin
length
acceptance
Health
UK
blood
Energy
solids
meat
growth
weaning
Nutrition
nutrient
maternal
infants
eating
breastfed
diet
fruit
differences
variety
children
feeding

TABLE OF CONTENTS

List of Tables

List of Figures

List of main abbreviations

BLW	Baby-led weaning
BMI	Body Mass Index
BMJ	British Medical Journal
BMZ	BM- for-age z-score
CHO	Carbohydrate
COMA	Committee on the Medical Aspects of Food Policy
DINO	Diet In Nutrients Out
DRV	Dietary Reference Values
EAR	Estimated Average Requirement
EFSA	European Food Safety Authority
ESPGHAN	European Society of Paediatric Gastroenterology, Hepatology and Nutrition.
Hb	Haemoglobin
HNR	Human Nutrition Unit
LAZ	Length-for-age z-score
LRNI	Lower Reference Nutrient Intake
MUFA	Mono-unsaturated fatty acids
NSP	Non-Starch polysaccharides
OR	Odds Ratio
PUFA	Poly-unsaturated fatty acids
RNI	Recommended Nutrient Intake
SACN	Scientific Advisory Committee on Nutrition
UK	United Kingdom
WAZ	Weight-for-age z-score
WHO	World Health Organisation
WLZ	Weight-for-length z-score

Acknowledgements

I'd like to thank my supervisors Dr Imogen Rogers, Dr Pauline Emmett and Dr Angela MacAdam for their support during the past 4 years. Thank you for your encouragement especially when it all felt so difficult and overwhelming. Angela, thank you so much for keeping me sane and for helping me through the emotional rollercoaster of the final weeks before submission.

To the mums of the Brighton's Breast-fed Babies Study, this thesis would not have been possible if it wasn't for you. Thank you for welcoming me in your homes and for being so helpful throughout the 12 months of the study and beyond. A special thanks to the 20 babies, (who will have started school this year!), you were my very first research participants and I will never forget you.

Thanks to everyone who helped me with my training: the research nurses from the Cambridge Baby Growth Study team (heel prick and anthropometric measurements training), Dr Heike Rabe and Denise Stilton from the Royal Sussex County Hospital (Haemospect provision and training) and Phil Matthews and Girish Ravindra from the Nuffield Hospital in Brighton (Haemoque provision and training). A special thanks to staff at the Human Nutrition Research Unit in Cambridge and especially Dr Celia Greenberg and Dr Emily Fitt for providing training and support for the nutrient analysis. Also, I'd like to thank researchers of the GO-CHILD study team and especially Professor Somnath Mukhopadhyay for providing me with the GO-CHILD questionnaires. Thanks to Dr Sarath Ranganathan for offering his guidance and advice on clinical aspects of the Brighton's Breast-fed Babies Study.

I'd also like to thank Matt Homer, Claire Ryan and Sarah Flux from the School of Pharmacy and Biomolecular Sciences for their help, especially in the past couple of years when I was not based at the university. Alex and Paul from reprographics, you have always been so helpful and efficient, thanks for everything (including printing this thesis!).

A special thanks to my friends and family for all their love and support. To all my PhD student colleagues in H402, thanks for reminding me that the PhD does not have to be a lone journey, it's been a privilege knowing you all. Meenal, Nina, Sharon and Sarah, it's been so sweet of you to keep an eye on me every day and to encourage me to keep going throughout this difficult period. A big thank you to my UCL colleagues who have also been so brilliant in keeping me motivated to work on the PhD on a daily basis.

To my “UK parents”, Mike and Margaret Moss, you have been a constant rock of support throughout the PhD and in the past 9 years. I wouldn’t have been in the UK if it wasn’t for your generosity and kindness.

A special thanks to Professor Richard Watt, Dr Georgios Tsakos and Professor Aubrey Sheiham, from the Department of Dental Public Health at UCL. Your support, kindness and understanding have been truly amazing. I cannot thank you enough for everything that you have done for me.

To my dad and my brother, thank you so much for your love and for always being here for me. I wouldn’t have done it without you.

To my mum, whose untimely passing a few weeks before the start of the PhD devastated me and our family: We started this journey together, and even though I had to continue without you, your words of advice, your belief in me and your encouragement have been with me all the way.

To my husband Anthony, words cannot express my gratitude for your unconditional love and support through these really challenging years. Thank you for always believing in me (even when I didn't believe in myself), for waking up at 4am to make me cups of tea whilst I was writing up, for making sure I ate properly, for putting your life on hold for so long for me. You have been my strength and I couldn't have done this without you by my side.

Declaration

I declare that the research contained in this thesis, unless otherwise formally indicated within the text, is the original work of the author. The thesis has not been previously submitted to this or any other university for a degree, and does not incorporate any material already submitted for a degree.

Antiopi Ntouva

Chapter 1

General Introduction

1 General Introduction

1.1 Setting the scene

Much has been written about the short-term and long-term benefits of exclusive breastfeeding for the first 6 months of life, both to the infant and the mother (Kramer & Kakuma 2012; Ip et al. 2007; Kramer & Kakuma 2002). Its undeniable benefits have resulted in many countries worldwide, including the UK, changing their public health recommendations from 4-6 months to 6 months of exclusive breastfeeding. However, there is still considerable debate as to whether the optimal age for introducing solids is indeed 6 months, especially in developed countries. This area of infant feeding research is not only relevant to paediatricians, dietitians, health visitors and other health professionals or policy makers; it is a subject that every parent and carer is very interested in, as they all want to ensure that they are equipping their infants with the best start in life. With the increasing rise of social media, a plethora of information of varying quality regarding the timing of solid food introduction is readily available to millions of parents looking for guidance and advice. Controversy in terms of advice in infant feeding practices is also evident in the scientific field, with experts from both sides of the argument debating about what the optimal age of introducing solids to infants should be. The matter is further complicated by the growing popularity of baby-led weaning (BLW), a complementary feeding method which, in addition to advocating introduction of solids at 6 months, promotes infant self-feeding with graspable, bite-sized foods (“finger foods”), instead of pureed or mashed foods fed with a spoon by parents.

Some of the key concerns raised about infants who are introduced to solids at 6 months, as well as those who follow BLW, include compromised growth as a consequence of nutrient inadequacy, impaired iron status and problems in accepting new flavours and textures, leading to eating difficulties such as fussy eating and limited variety of foods in the diet.

1.2 Problem statement

There is a paucity of studies in industrialised countries investigating the association between the 6 month solid food introduction recommendations and growth, diet, iron status and feeding problems both in the transitional period of complementary feeding (i.e. in the first 2-3 months after the first solid food is introduced), as well as when feeding has been established (i.e. 6-12 months after the first solid food is introduced). A cohort study in an infant population that adheres to the current recommendations could elucidate this transitional period of infant feeding by following changes in growth, nutrient intakes and iron status, as well as feeding behaviours as the infants' diet integrates with that of the family. Also, BLW has never been examined longitudinally before and therefore a study which follows up infants who are introduced to solids using this method could provide useful insights on the longer-term aspects of this feeding practice in this infant subgroup. Since infants and toddlers are at a higher risk of developing iron deficiency anaemia, validating a non-invasive method of measuring haemoglobin against current invasive practices in community settings, could result in the attainment of a very useful tool in identifying infants and toddlers with low haemoglobin levels quickly and easily and alert parents to seek further medical attention. Finally, examining dietary variety, food preferences and feeding problems in the early complementary feeding period using a cross-sectional study design, could provide a snapshot of the infants' feeding behaviours in the first months after the introduction of solid foods.

The thesis will aim to address the key issues outlined above, in light of current UK infant feeding recommendations.

1.3 Definition of key terms

Before proceeding further, it is necessary to define the key terms describing infant feeding practices of the study participants, which will be used throughout the thesis. Further definitions of other key concepts can be found in the relevant chapters.

For the purposes of this thesis, infants who are breast-fed either exclusively or with high breastfeeding intensity are defined as "principally breast-fed". An infant is characterised as exclusively breast-fed, when he or she is receiving solely breast milk with no other food or drink apart from drops or syrups containing vitamins, minerals or medicines. An infant is characterised as breast-fed with high intensity, when he or she is receiving breast milk for ≥80% of total milk feeds (Labbok & Krasovec 1990). Mixed-fed infants are defined as those drinking both formula and breast milk of medium (20-80) or low (<20%) or unknown intensity or when the proportion of breast milk is not known (Labbok & Krasovec 1990).Complementary feeding is the introduction of solid, semi-solid foods and drinks other than breast milk and formula milk.

According to Gill Rapley, the health visitor who defined the term baby-led weaning, it's "*a way of introducing solid foods that allows babies to feed themselves - there's no spoon feeding and no purées. The baby sits with the family at mealtimes and joins in when she is ready, feeding herself first with her fingers and later with cutlery"* (Rapley 2005).

Feeding problems such as fussy eating, establishing an eating routine, as well as food preferences and dietary variety will be broadly referred to as "eating behaviours".

Comparisons throughout the thesis will be made between infants who were introduced to solids before or after 6 months (referred to as the "early" and "late" complementary feeding groups) and between infants who follow BLW and those who are spoon-fed.

1.4 Purpose of thesis

The purpose of the thesis will be to address the following research questions:

- Are there any differences between infants who are principally breast-fed and introduced to solids at or after 6 months, on growth, diet, iron status and eating behaviours at 8 and 18 months compared to those introduced to solids before 6 months? Are there any differences in the above outcomes in the same infant population when stratified by mode of complementary feeding, i.e. those who followed baby-led weaning and those who were spoon-fed?

- How does the use of a non-invasive technique measuring haemoglobin levels in 18 month-olds compare to heel prick measurements?

- What is the association between the age of solids introduction in a sample of mixed-fed infants and eating behaviours, food preferences and dietary variety at 9 months?

Detailed aims and objectives for the research questions described above will be presented in the following chapters.

1.5 Original contribution to knowledge

Following a review of the literature in Chapter 2, the thesis will aim to add to the existing evidence on timing of solids introduction and growth, diet, iron status and eating behaviours in the following ways.

Firstly, to our knowledge, there have been no studies in the UK since the adoption of the current infant feeding recommendations assessing growth, diet, iron status and eating behaviours in infants introduced to solids at 6 months both in the transitional complementary feeding period as well as when infants' diet patterns have been established. For another, studies on BLW have all been either qualitative or cross-sectional and there are no studies up to now that have looked at detailed nutrient intakes, growth, iron status and feeding problems in infants following BLW longitudinally. In Chapter 3, infants from Brighton and Hove who follow the 6 months recommendation for infant feeding, will be compared with those who were introduced to solids before 6 months and their growth, food and nutrient intakes, iron status and feeding behaviours will be assessed. These outcomes will also be examined by stratifying the infants by mode of complementary feeding (BLW and spoon-fed).

Despite the calls for universal screening for iron deficiency anaemia around 18 months of age and the associated complications with invasive methods of blood taking, a non-invasive measurement of haemoglobin has never been tested before in 18 month olds in the UK. In Chapter 4, a non-invasive device measuring haemoglobin levels in a subgroup of 18 month old infants will be validated against measurements taken from a heel prick blood sample.

There have been no studies in the UK looking at timing of solids introduction at 6 months and dietary variety, food preferences and associated feeding problems in the early complementary feeding period. In Chapter 5, the association between the age of solids introduction and eating behaviours will be assessed in a sample of 9 month old infants from Sussex.

Finally, in Chapter 6, the extent to which these gaps in the literature have been successfully addressed will be discussed and the main outcomes of each study will be placed within the wider context of other research as well as clinical practice, acknowledging the limitations of each study and identifying areas for future research.

Chapter 2

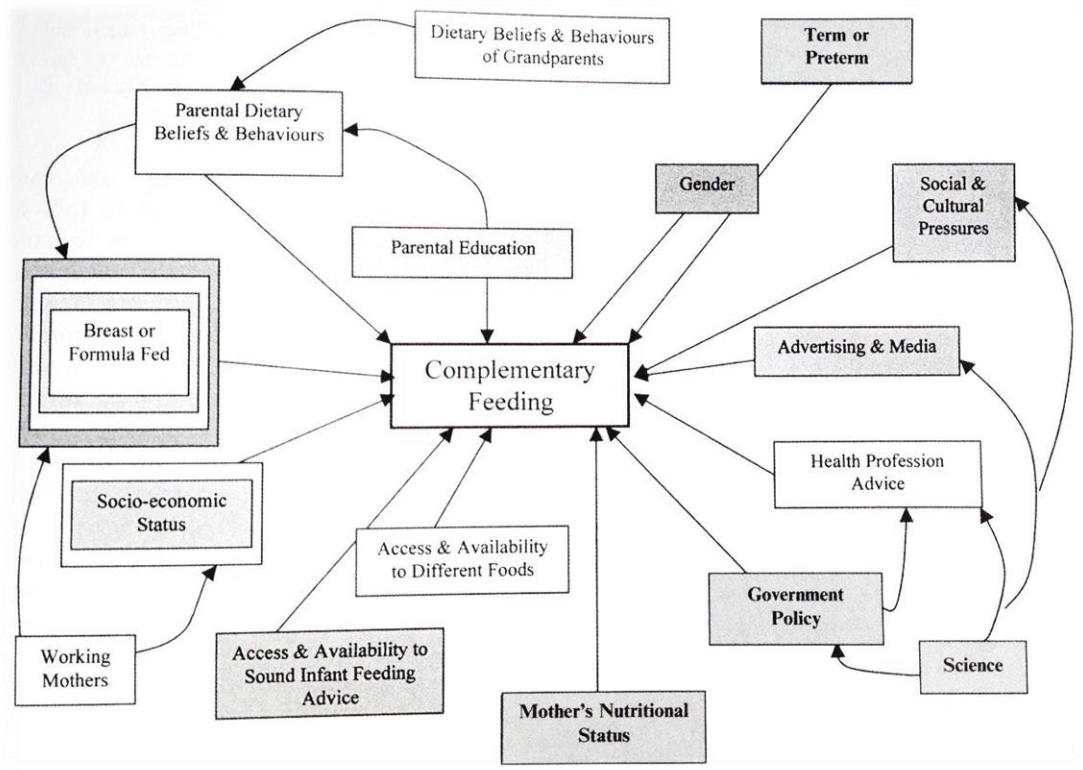
Literature Review

2 Literature Review

2.1 Chapter Overview

Complementary feeding practices are influenced by a complexity of factors (Figure 2.1). This chapter will review the existing evidence in the literature on the association between early versus late introduction of solids foods as well as BLW versus spoon feeding (as defined in Chapter 1) with growth, diet, iron status and eating behaviours. The review focused on studies that were published following the World Health Organisation's (WHO) recommendations on exclusive breastfeeding in 2001 in developed countries and especially the UK, although some key studies from developing countries that were published before 2001 have also been included.

Figure 2-1 Factors that influence complementary feeding practices



(Morgan & Dickerson 2003)

2.2 Reviews and reports on timing of solids introduction: 4-6 versus 6 months

Although the review by Kramer and Kakuma and subsequent updates focused on the benefits of exclusive breastfeeding for six months as opposed for 4-6 months (Kramer & Kakuma 2002), Lanigan and colleagues performed the first systematic review in 2001 to assess the appropriateness of the then WHO recommendations for solids introduction at 4-6 months versus 6 months (Lanigan et al. 2001). They identified 33 out of 400 journal articles between 1982 and 1998 in which age of complementary feeding and one of the outcomes of growth, nutritional status or morbidity were mentioned. Due to widespread heterogeneity across the studies, it was not possible to synthesize the extracted data and the lack of population specific reference data to identify inadequate nutritional status, made it impossible to draw safe conclusions. The issue of reverse causality was raised, as well as the fact that for certain subgroups of infants (such as those born prematurely), delayed solids introduction by prolonged exclusive breastfeeding may not be appropriate. The authors ultimately concluded that there was not enough good quality evidence in favour or against the change in the recommendations to 6 months. This systematic review is relatively out of date and the majority of studies included were from developing countries. In the nine studies from developed countries (UK, USA, Finland, New Zealand) that were included in the review, there were 3 studies from the UK, one of low methodological quality [the sample size was not presented, boys and girls were examined using different cut-offs for solids introduction (16 weeks for boys and 20 weeks for girls) and it was unclear if results were crude or had been adjusted for confounding factors]. The remaining 2 studies, derived from the same cohort study in Scotland, used 12 and 15 week cut-off points to define early and late solids introduction. None of the studies examined in developed countries were designed to assess outcomes between those introduced to solids between 4-6 months and those introduced to solids at or after 6 months. Finally, the authors were criticized for having received money from the infant food industry in the past, even though the study was claimed to be independently conducted. It was therefore not possible to extract conclusions about the effect of the timing of solids introduction in a UK population based on this review.

A review by Dewey noted that the advantages of introducing solids at 6 months in developed countries may be less evident than those in developing countries but in healthy, full-term infants, the evidence suggests that although there may be no benefit there is also very little risk in introducing solids at 6 months with regards to growth, food acceptance and iron and zinc status. She did however call for more studies looking at the of the age of solids introduction in industrialized countries (Dewey 2006).

After conducting a systematic review of assessing energy content in breast milk and its transfer rate from mother to infant in the first six months of life (Reilly et al. 2007), Reilly and Wells went on to form the assumption that breast milk would not fulfil energy requirements of the exclusively breast-fed infant at 6 months, based on theoretical calculations (Reilly & Wells 2007). However, the calculations of energy requirements were based on the 1990 UK growth charts which are well known to misestimate the growth of breast-fed infants as they were based on formula-fed infants. In fact, a later study from some of the same authors (Nielsen et al. 2011), concluded that compared to the WHO growth standards, exclusive breastfeeding in healthy mothers and infants was sufficient to support growth up to 6 months.

The European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) commentary paper on complementary feeding, acknowledged that the debate over the duration of exclusivity of breastfeeding overshadowed the potential effect of the appropriate age of solids introduction and that there was lack of evidence in industrialised countries in that respect (Agostoni et al. 2008). Its main recommendation was for mothers to aim to breastfeed exclusively for 6 months but the committee still recommended a 4-6 months window for solids introduction (Agostoni et al. 2008). Although other aspects of health were discussed, the timing of solids introduction was only considered in the light of disease prevention in terms of allergy, coeliac disease and diabetes mellitus, perhaps reflecting the nature of the panel, as the vast majority consisted of paediatricians and gastroenterologists. Although neurodevelopmental readiness for receiving solids more efficiently at 6 months compared to 4 months was acknowledged, it was not taken into consideration in the final recommendations on introduction of solids. Finally, food preferences and nutritional adequacy were not reviewed in the light of timing of complementary feeding before and after 6 months.

A year later, the European Food Safety Authority (EFSA) reviewed the available evidence with regards to the age of solids introduction (EFSA Panel on Dietetic Products 2009). Initially it was acknowledged that breast milk is nutritionally adequate for up to 6 months;

however it noted the lack of parallel nutrient and anthropometric data after 6 months. The report followed a similar line of arguments to the one by ESPHGAN and for most cases such as growth, food preferences, obesity, type 2 diabetes, allergy and infection there was either limited, or no strong evidence of an association with the timing of solids introduction. Its final recommendation however was that introducing solids between 4-6 months is safe for both short-term and long-term health outcomes. There was no information of the methods used to extract the relevant literature or how the data from the studies quoted in the report fared in terms of quality.

A review paper by Fewtrell and colleagues in the British Medical Journal (BMJ) in 2011 asking for the existing guidelines for solids introduction at 6 months to be revised (Fewtrell et al. 2011), sparked an intense debate both in the media as well as the scientific community with advocates on both sides of the argument speaking out. Fewtrell et al reviewed the literature selectively, did not provide a clear search strategy of the literature cited and did not rate the strength of the evidence provided. There were potential conflicts of interest due to the authors' associations with the infant food industry as well. Finally, the studies quoted were already known to the scientific community and therefore the paper did not add to the existing knowledge.

The ESPHGAN commentary paper was criticized in 2011 by Cattaneo and colleagues (Cattaneo et al. 2011) for the lack of a systematic approach to the retrieval, analysis and interpretation of the literature used as well as conflicts of interest, as ESPHGAN and some of its authors have been receiving funding or other support from the infant milk and infant food industry. The authors concluded that the ESPHGAN report as well as the EFSA report (which shared the main author) should be treated as expert opinions, which provide the lowest level of quality in evidence-based medicine. Cattaneo et al. proposed that the developmental readiness of the infant to cope with foods other than milk should be taken into account, when assessing the appropriate age to introduce solids. The authors went on to describe the concept of a "window of achievement", a time period within which the combination of neurodevelopment of reflexes as well as immune, renal and gastrointestinal maturation allows the infant to safely ingest solid food. Although there is no certainty about the exact timeline, it should be treated as any other developmental milestone and include a range of ages rather than one particular time point. They described this range as a negatively skewed bell-shaped curve with the mode around 6 months and the tails between

4 and 10 months. Therefore, they suggested that most infants would be ready for solid foods around 6 months and very few at around 4 months. The authors concluded that the concept of the developmental achievement window compliments the current infant feeding recommendations. However, due to ethical constraints, the existence of such window cannot be experimentally verified and therefore the concept remains fairly empirical and theoretical.

In summary, reviews comparing the timing of solids introduction before and at/after 6 months, provided very mixed results due to lack of good quality original studies from the developed world, selective review of the available evidence as well as potential conflicts of interest of some of the authors with the infant food industry.

The next sections will explore the evidence on the of age of solids introduction and mode of complementary feeding on growth, nutrient adequacy, iron status and eating behaviours.

2.3 Timing of solids introduction and growth

2.3.1 Infant growth patterns before the introduction of solids

Infant growth depends primarily on what and how much infants are being fed and the rate at which they grow and is an important determinant of later disease risk (Weaver 2012). Birth weight is a general index of the infant's intrauterine growth and subsequent growth measurements summarise the infant's overall development. There are marked differences in growth rates between breast-fed infants and formula-fed infants, primarily due to differences in the composition of the milks and the amounts taken. Breast-fed infants lose more weight over the first few days after birth than bottle-fed infants (median 6.6% for breast-fed over 3.5% for bottle-fed, $P<0.001$) and they regain the lost weight later than bottle-fed infants (median 8.3 days for breast-fed versus 6.5 days for bottle-fed, $P<0.001$) according to Macdonald and colleagues (Macdonald et al. 2003)**.** One of the reasons for this could be the time required to establish milk production and a regular breastfeeding routine between the mother and the newborn infant. Most infants reach their nadir weight (i.e. the lowest weight before weight gain begins) between day 2-3 post-partum (Grossman et al. 2012). After the first week of life and up to 2-3 months, growth rates tend to level between breast-fed and formula-fed infants for both weight and length (Butte et al. 2000; Dewey et al. 1992; Koletzko et al. 2009). After 3 months however, growth patterns begin to deviate again between the 2 groups of infants. Results from the Generation R study in the Netherlands, showed that between 3-6 months of age, duration and exclusivity of breastfeeding was inversely associated with weight-for-age and length-for-age z-scores (Durmuş et al. 2011). Growth patterns during infancy are also reflected in energy requirements in this period; the proportion of energy required for growth is very high in the first 3 months (35% of total energy requirements), then drops to half in the next 3 months (17.5%), 3% at 12 months and 2% in the second year of life (Scientific Advisory Committee on Nutrition) (Scientific Advisory Committee on Nutrition 2011b).

The UK 1990 growth charts were based primarily on formula-fed or mixed-fed infants and therefore the slower growth of breast-fed infants (which was evident as "dropping centiles" on the chart) was perceived as growth faltering from the second half of infancy. By 12 months, breast-fed infants were lighter by as much as half a centile compared to formula-fed infants (SACN 2007).

Recognising the difference in the growth rate of breast-fed infants, the WHO published new growth standards based on longitudinal data (24 months follow up) from 6 countries (Brazil, Ghana, India, Norway, Oman and USA) and cross-sectional data (infants aged 18-71 months) of infants growing up under optimal conditions, exclusively or predominantly breast-fed for at least 4 months (in the longitudinal study) or 3 months (in the cross-sectional study) and introduced to solids by 6 months. The organisation's evaluation of the infant growth found that breast-fed infants were much leaner than formula-fed infants; however, this did not represent a faltering in growth. From 2-12 months weight-for-age z-scores were declining whereas length-for-age z-scores declined earlier and plateaued or slightly increased at around 8 months (World Health Organisation 1995). Z-scores are a way of standardising raw data and are calculated by subtracting the raw value from the population mean and then dividing this by the standard deviation of the mean ($z = (x-\mu)/\sigma$). The resulting score represents the number of standard deviations that the measurement deviates from the mean (in this case, the growth standards used). A negative z-score indicates a data point below the average; a positive score indicates a data point above the average. A small z-score indicates that a data point close to the mean.

The main difference between the growth references previously used and growth standards is that growth references reflect the current state of the population and hence can change over time and do not necessarily represent the optimal growth. Conversely, the new growth standards are based on normal growth among a population of infants fed according to current infant feeding recommendations. In 2007, SACN recommended the adoption of the WHO growth standards in the UK from the age of 2 weeks onwards (to account for weight adjustments during the first 2 weeks post-partum, differences in birth weight and gestational age (Scientific Advisory Committee on Nutrition 2007a). In 2009, the WHO standards were introduced in clinical practice in the UK and are currently used to assess growth from birth to 4 years (Scientific Advisory Committee on Nutrition 2011b). They represent a growth pattern under ideal conditions, whereby growth is not restrained by environmental factors (Ziegler & Nelson 2010).

According to a review by Ong and Loos (Ong & Loos 2006) there is strong epidemiological evidence to suggest that in healthy full-term infants, rapid weight gain increases the risk of obesity in later life by 2-3 fold and to a lesser extent the risk of heart disease and other metabolic consequences (Belfort & Gillman 2013). Data from systematic reviews and meta-analyses continue to demonstrate a protective effect of breastfeeding against later obesity (Koletzko et al. 2013). Data from the IDEFICS study, which examined the role of exclusive breastfeeding on measures of overweight and obesity in 14726 children aged 2-9 years from 8 European countries found that, after controlling for socioeconomic factors and maternal overweight, breastfeeding exclusively for 4-6 months was protective of overweight and obesity (OR = 0.73; 95 % CI 0.63, 0.85). Also, exclusively breastfeeding for 6 months offered a marginally higher protection than breastfeeding for 4 and 5 months (OR = 0.71; 95 % CI 0.58, 0.85) (Hunsberger et al. 2013).

Even though there is sufficient evidence concerning the protective role of breastfeeding against rapid weight gain and future metabolic consequences, there is still relatively little information about the effect of the age of solids introduction in the growth patterns of infants both in the short-term and long-term.

2.3.2 Infant growth patterns after the introduction of solids

There is still lack of evidence with regards to the age of solids introduction and growth especially in terms of the risk of overweight and obesity in later life (Lanigan et al. 2001). Studies examining the age of solids introduction and anthropometric measurements provide mixed results. Most studies examining the effect of the complementary feeding period on growth, have compared either just formula-fed infants or breast-fed versus formula-fed infants. Results from the DARLING study indicate that there are large differences in indices of body fatness between breast-fed and formula-fed infants, especially during the later complementary feeding period of 9-15 months of age. Feeding mode was no longer significant after adjusting for energy intake, however formula-fed infants had consistently higher energy intakes than breast-fed infants (Dewey et al. 1993). Data from 5 observational studies in the UK of formula-fed infants reviewed by Morgan and colleagues showed that those introduced to solids earlier were larger at 12 weeks than those introduced to solids later. However, by 18 months there were no differences in growth parameters between groups (Morgan et al. 2004).

A randomised trial in the Honduras showed that the age of solids introduction did not influence growth patterns up to 6 months of age in 3 groups of infants; those exclusively breast-fed for 6 months, infants who were introduced to solids at 16 weeks and maintained the pre-intervention breastfeeding frequency and those who were introduced to solids with ad libitum breastfeeding. Although the infants grew at a faster pace between birth and four months, their growth patterns from 4-6 months were similar to an American sample of breast-fed infants (Cohen et al. 1994). However the study had a high dropout rate (66%) and the solids introduced were commercially prepared foods provided by the researchers. It could be possible therefore that participants changed their behaviour in terms of frequency of feeding because of the study. Breastfeeding frequency was also significantly higher at home compared to the time the mothers spent in the clinical investigation unit during data collection for the study. Also there was no further information about the growth of the infants who exclusively breast-fed for six months beyond that time point when solids were introduced. In a subgroup of 60 breast-fed infants from the DARLING study, Heinig and Nommsen found that those in the later solids introduction group (26 weeks or later, n=19) had a slower weight gain rate (but not length gain rate) than those in the early solids introduction group (16-25weeks, n=41) from 6-9 months. There was no significant association between age of solids introduction and weight and length from 1 to 18months (Heinig & Nommsen 1993). It is unclear how many of the infants in the study were exclusively breast-fed as the inclusion criteria called for infants in the breast-fed group to not consume more than 120ml/day of other milk or formula in the first 12 months of the study.

Baird et al using data from 1740 infants from the Southampton Women's survey showed that infants who were introduced to solids before 5 months were heavier and longer at 6 months than those introduced to solids at 5 months or later. However the growth of the later complementary feeding group did not differ from the earlier group of infants with or without adjustment for milk feeding in the first 6 months. During the second half of infancy (6-12 months) those whose diet was closer to the infant feeding guidelines (homemade foods, fruit and vegetables, continuation of breastfeeding) grew more rapidly in weight, length and adiposity than those with a diet of processed foods. All the associations were independent of maternal education, parity and smoking during pregnancy (Baird et al. 2008). Data from the same cohort did not show an association between age of solids introduction and BMI at four years of age, after adjusting for confounding factors (Robinson et al. 2009).

A study by Sloan et al followed 210 infants from birth to 14 months. Infants were categorised as early weaned (before 4 months) versus late weaned (after 4 months). Those who were introduced to solids early had significantly higher weight-for-age z-scores at 7 months (p=0.005) and 14 months (p=0.04) and increased weight gain rate between 8weeks and 14 months (p=0.003). When duration of breastfeeding was taken into account, the relationships were slightly attenuated but still remained significant (Sloan et al. 2008). However, duration of breastfeeding in this study was defined as any breastfeeding regardless of the introduction of other milks, formula milk or solids. Breastfeeding was initiated by 68% of the sample (n=148) and by 6 months only 42 infants were still breast-fed. Therefore it is unclear what effect exclusivity of breastfeeding had on growth in this group.

Results from the Viva La Familia study, which looked at the effect of genetic and environmental factors on obesity, did not find any association between obesity and exclusive breastfeeding (p=0.58) or age of solids introduction (p=0.6) in a group of 1030 Hispanic children. The authors argued that although breastfeeding is protective, other factors such as socioeconomic status and rapid weight gain are more important risk factors for obesity in childhood (Butte 2009). Only a third of the sample had breast milk as the only source of milk and although there was information on the exclusivity there was no information on its duration and effect. Moreover, information on infant feeding practices was collected retrospectively (as the eligibility criteria was children aged 4-19 years) and therefore there is the potential for a wide variation of recall bias in terms of infant feeding practices.

Examining the combined effect of breastfeeding and complementary feeding has produced mixed results. Burdette and colleagues did not find significant associations between adiposity and duration of breastfeeding and age of solids introduction (before and after 4 months) after adjusting for confounding factors such as socioeconomic status, maternal obesity and smoking (Burdette et al. 2006). A study by Baker and colleagues found that infants who were breast-fed for longer and were introduced to solids later had slower weight gain rates by 1 year of age. Pre-pregnant maternal BMI explained some of the same variance in infant weight gain (Baker et al. 2004). However due to the questionnaire design, it was not possible to establish when exclusive breastfeeding ceased.

The associations between the risk of obesity and the age of complementary feeding, exclusivity and duration of breastfeeding and formula feeding are not clear. A recent literature review by (Moorcroft et al. 2011) which reviewed 24 studies (34,000 participants), examined the association between age of solids introduction and risk of obesity in early (<12 months) and later life (1-18 years); it included studies with breast-fed and formula-fed infants but did not find consistent associations. However, there was some evidence of self-regulation of energy intake in breast-fed infants, not only in the first months of life, but also during the complementary feeding period, when compared to their formula-fed counterparts (Nommsen-Rivers & Dewey 2009) which warrants further investigation. Higher intakes in mixed-fed and formula-fed infants from the ALSPAC study predicted greater gain at ages 1,2 and 3 years and higher BMI between 1-5 years, something that was not observed in breasted infants (Ong et al. 2006). Even though breast-fed infants had lower BMI than formula-fed infants, the differences were not very large and could be due to confounding factors (Owen et al. 2005).

There is a wide variation in the results from studies looking at the effect of age of solids introduction and growth, in terms of using different cut-off points (with the majority using before/after four months), settings (developed/developing world), timing of collection of infant feeding information (most studies did this retrospectively), confounding factors adjusted for, as well as study end points (ranging from a few months post-partum until adulthood). Most of the studies were observational, with small to medium sample sizes and the majority did not include information on both duration and exclusivity of breastfeeding.

2.3.3 Infant growth patterns and baby-led weaning

The popularity of baby-led weaning is growing, as it is seen as a more natural approach to infant feeding. In the latest infant feeding survey 4% of mother reported that the first solid food introduced to their infants was a finger food (McAndrew et al. 2012b). However, the extent to which infant self-feeding with finger foods may be affecting growth, especially in the immediate complementary feeding period is yet to be investigated. There has only been one study so far looking at growth and baby-led weaning, which was based on parental self-report of weight and length, which was collected retrospectively (20-78 months) and was compared to growth measures taken by the researchers from a group of spoon-fed infants. The researchers found higher rates of underweight in the baby-led weaned group and higher rates of overweight in the spoon-fed (Townsend & Pitchford 2012). However, apart from the self-reported growth measures in the baby-led group, there were additional methodological issues in the study. The sample was small, self-selected and the two groups were not recruited in the same way: the spoon-fed group was selected through a university database, whilst the baby-led weaned group was recruited via the internet.

2.4 Timing of solids introduction and diet

2.4.1 Nutrient requirements in infants and toddlers

In 1991 the Committee on Medical Aspects of Food Policy (COMA) devised a set of reference values for nutrient intakes for the UK population (Dietary Reference Values, DRVs). DRVs have been used ever since to assess the adequacy of population nutrient intakes by age group. Nutrient reference values fall into three categories: The Estimated Average Requirement (EAR) which is an intake sufficient for 50% of the population, the Reference Nutrient Intake (RNI) defined as two standard deviations above the EAR and therefore intakes above that would be adequate for 97.5% of the population and the Lower Reference Nutrient Intake (LRNI), which is two standard deviations below the EAR, represents the lowest intakes which will meet the needs of approximately 2.5% of individuals in the group. In practice, this means that if a group's mean nutrient intakes are above or equal to the RNI, the risk of deficiency in that group is likely to be small. If however the mean nutrient intakes of a group are below the LRNI, there is a great risk of nutrient deficiency. RNIs and LRNIs for protein, vitamins and minerals at ages 8 and 18 months are shown in Table 2-1.

Looking at iron requirements in particular, COMA recognised that infants and toddlers are vulnerable to iron deficiency due to their high iron requirement for growth, especially in the complementary feeding period where late weaning and/or inappropriate weaning foods may accentuate the problem. Iron requirements were estimated taking into consideration iron losses, growth requirements, and dietary bioavailability. In breast-fed infants it was assumed that 50% of breast milk iron is absorbed as opposed to 10% of dietary iron in formula-fed infants up to 3 months of age. At ages above 3 months, an iron absorption of 15% was used to calculate the dietary reference values (DRV) (Committee on Medical Aspects of Food Policy (COMA) 1991). SACN's report on iron and health highlighted the lack of good quality data in terms of uptake and losses, physiological adaptation to low systemic iron and iron stores (Scientific Advisory Committee on Nutrition 2010). Therefore the estimates on iron requirements may be too conservative and overcautious. There are also questions regarding the necessity of setting of dietary iron requirements for the first six months of life as the body relies upon its endowment from birth to meet requirements in this time period (Scientific Advisory Committee on Nutrition 2010).

Table 2-1 Dietary Reference Values (DRVs) for energy and nutrients by age

Nutrient (unit of measurement) per day	**DRVs**	**Age**	
		8months	**18 months**
Energy (KJ/kg/d)[a]	EAR (boys)	330	-
	EAR (girls)	328	-
Energy (kcal /kg/d)[a]	EAR (boys)	79	-
	EAR (girls)	78	
Energy (KJ/d)[a]	EAR (boys)	-	370
	EAR (girls)		345
Energy (kcal /d)[a]	EAR (boys)	-	884
	EAR (girls)	-	824
Protein (g/d)[b]	RNI	13.7	14.5
Thiamine (mg/1000kcal)[c]	RNI	0.30	0.40
	LRNI	0.20	0.23
Riboflavin (mg/d)	RNI	0.40	0.60
	LRNI	0.20	0.30
Niacin (mg niacin equivalents./1000kcal)[c]	RNI	6.6	6.6
	LRNI	4.4	4.4
Vitamin B6 (µg/g protein)[d]	RNI	10.0	15.0
	LRNI	6.0	11.0
Vitamin B12 (µg/d)	RNI	0.4	0.5
Folate (µg/d)[b]	RNI	50	70
	LRNI	30	35
Vitamin C (mg/d)	RNI	25.0	30.0
	LRNI	6.0	8.0
Vitamin A (retinol equivalents) (µg/d)	RNI	350	400
	LRNI	150	200
Vitamin D (µg/d)[b]	RNI	7.0	7.0
Calcium (mg/d)	RNI	525	350
	LRNI	240	200
Phosphorus (mg/d)	RNI	400	270
Magnesium (mg/d)	RNI	75	85

Nutrient (unit of measurement) per day	DRVs	Age	
		8months	18 months
	LRNI	45	50
Sodium (mg/d)	RNI	320	500
	LRNI	200	200
Potassium (mg/d)	RNI	700	800
	LRNI	400	450
Chloride	RNI	500	800
Iron (mg/d)	RNI	7.8	6.9
	LRNI	4.2	3.7
Zinc (mg/d)	RNI	5.0	5.0
	LRNI	3.0	3.0
Copper (mg/d)[b]	RNI	0.30	0.40
Selenium (µg/d)	RNI	10	15
	LRNI	5	7
Iodine (µg/d)	RNI	60	70
	LRNI	40	40

The age groups presented are those for which different RNI and LRNI values have been calculated. DRVs are presented as 8 and 18 months. The COMA age groups were 7-9 months and 1-3 years however for the purposes of this study they are represented this way for clarity.

Department of Health. Dietary Reference Values for Food Energy and Nutrients for the United Kingdom. London. HMSO. 1991. [Report on Health and Social Subjects: 41]

Abbreviations: EAR: Estimated Average Intake, RNI: Reference Nutrient Intake, LRNI: Lower Reference Nutrient Intake, KJ: Kilojoules, Kcal: kilocalories, d: day, g:gram, mg: milligram, µ: microgram

[a]Calculation based on body weight and age in months (up to 12 months) and years (aged 1 year and over).For children aged under one year, energy intakes are compared with the mixed or unknown feeding group for estimated average requirement (EAR) for energy. For those aged 12 to 18 months, intakes are compared to the physical activity level (PAL) adjusted for growth for boys and girls (SACN, 2011)

[b] There is no LRNI for protein, Folate, Vitamin D and Copper (COMA 1991)

[c] Calculated values based on Estimated Average Requirement (EAR) for energy; calculated values from quoted LRNIs mg/1000kcal.

[d] Based on protein providing 14.7% of the EAR for energy; calculated values from quoted LRNIs µg/g protein.

2.4.2 Nutritional adequacy after the introduction of solids

Studies on nutritional adequacy of complementary feeding have mainly focused on developing countries (Cohen et al. 1994; Batal et al. 2010) or at-risk infant populations, such as those born prematurely (King 2009; Fanaro et al. 2007) or those born to HIV infected mothers (Becquet et al. 2006). In the case of developing countries, there are many extrinsic factors that can affect the provision of nutrients in the infant's diet, such as food security, food availability and hygiene which do not apply to infants from developed countries. In terms of preterm infants, due to the fact that acquisition of several nutrients such as iron occurs during the last trimester, their nutritional requirements deviate markedly from those born to term and therefore need different nutritional interventions. Nutritional adequacy of breast milk from a well-nourished mother to a term infant should be sufficient until 6 months for most nutrients apart from Vitamin D (due to the minimal if any sun exposure to the sun) and zinc, as its concentration in milk declines after 4-5 months irrespective of maternal zinc status (Foote & Marriott 2003).

A growing number of studies have been conducted to assess the effect of complementary foods and age of introduction in the infant's diet on the development of food allergies (Mihrshahi et al. 2007; Zutavern et al. 2008; Nwaru et al. 2010) and coeliac disease (Ludvigsson & Fasano 2012; van Odijk et al. 2004) with conflicting results regarding the timing of introduction of certain solids foods and increased risk of allergy sensitisation, eczema or coeliac disease. However, there is a lack of studies looking at nutrient intakes in the early complementary feeding period (8-9 months). The Southampton Women's Survey research group compared infants' diets at 6 and 12 months using a food frequency questionnaire and a 4-day weighed diary. Both methods showed reasonable agreement for most nutrients, however there was wide variation in intakes of energy and calcium for breast-fed infants (28% of the sample) compared to formula-fed infants. Exclusivity of breastfeeding was not defined and median age of solids introduction for the whole sample was 17.4 weeks (Marriott et al. 2008).

Principally breast-fed infants constitute a subgroup that could be a cause particular concern because, as they tend to be introduced to solids later, they don't receive fortified milk, which is the main source of several nutrients at this time point. For example, data from the Diet and Nutrition Survey of Infants and Young Children (which only identified 2 exclusively breast-fed infants out of 2683 infants and toddlers) showed that 57% of daily zinc intakes and 56% of daily iron intakes in 4-6 month old infants came from infant formula (Lennox et al. 2013). As a result, principally breast-fed infants could be more susceptible to nutritional deficiencies in the immediate period after 6 months where breast milk alone is not sufficient to meet the growing requirements for nutrients and the transition to solids foods is not yet fully established.

The ALSPAC team has produced a valuable insight into diets of infants at 8 and 18 months by using a large sample size and focusing on infant feeding practices including type of milk feeding and age of solids introduction. Noble and colleagues (Noble et al. 2001) looked at energy and nutrient intakes in a subset of the ALSPAC study population and compared the findings with the latest (at the time) national diet and nutrition survey for infants by Mills and Tyler (1996). Results for breast-fed infants (n=260) showed that they had significantly lower intakes of energy, protein, carbohydrate, vitamins and minerals than infants who were not breast-fed (n=871, p<0.01) before and after controlling for gender. In terms of food intakes, there were high intakes of whole cow's milk (mean 226g among 49% of sample) and commercial infant foods (146g, 74% of the sample). The 18 month follow up of the ALSPAC children showed that intakes of vitamins and minerals were within the recommended nutrient intakes for that age group. However, mean iron intakes were just below or around the Recommended Nutrient Intakes (RNI) (5.5mg/day for boys and 5.2mg/day for girls) and the median intake was below the Estimated Average Requirement (EAR) for that age group. Intakes of vitamin D were also very low and zinc intakes were marginal (Cowin & Emmett 2001). This data is consistent with figures from the rolling national diet and nutrition survey which have also indicated that diets of toddlers are nutritionally adequate and there is little cause of concern apart from Vitamin D and to a lesser extent iron (Bates et al. 2012). However the 1.5 year age group studied was incorporated into the 1.5-4 year age group and was not looked at separately due to its small sample size, therefore it is difficult to ascertain whether potential low intakes of nutrients at 1.5years were masked by higher intakes from older children.

Up until recently, there were no data on nutritional intakes on a nationally representative sample in the UK after the adoption of the WHO infant feeding guidelines by the Department of Health, until the publication of the national diet and nutrition survey for infants and young children in 2013 (Lennox et al. 2013). The report provides quantitative information of infant diets from 4-18 months but out of 2683 infants only 2 were exclusively breast-fed between the ages of 4-6 months (Lennox et al. 2013) and analysis did not include stratification by age of solids introduction.

A recent blinded randomised controlled trial in Iceland looking at iron status in infants exclusively breast-fed for 6 months and those introduced to solids at 4 months also examined selected nutrient intakes from complementary foods only at 5 ¼ months. Mean intakes of complementary food were very small (92.2 ± 66.5 g) and provided 10% of RDI (recommended diet intake), protein 18%, calcium 6% vitamin C 44% and vitamin D 43% (Jonsdottir et al. 2012). In a different study by the same research group, it was shown that the complementary feeding group consumed significantly less milk (818 ± 166ml) than the breastfeeding group (901±158ml, p=0.012). Although breast milk intakes in this study were consistent with WHO recommendations, energy intakes for both groups were below the WHO guidelines for energy requirements (Wells & Jonsdottir 2012). Several other "problem nutrients" have been identified which are relevant in both developed and developing settings. These include iron, zinc, calcium, Vitamin A in some developing countries (Brown 2007) as well as vitamin B12 and riboflavin (Foote & Marriott 2003).

2.4.3 Nutritional adequacy and baby-led weaning

Research in baby-led weaning practices has only just started emerging and there have been no studies looking in detail at nutrient intakes in BLW infants and whether they are comparable to infants who were not following the baby-led weaning approach. Rowan and Harris (Rowan & Harris 2012) looked at a proxy measure of assessing intakes of infants by comparing the diet of 10 parents of infants who were just starting to consume solid foods using the baby-led weaning approach with the types of foods offered to their infants to assess similarities between the 2. The foods that infants were being offered were in fact foods that at least one of the parents was also consuming, confirming that one of the principles of BLW (eating family foods) was followed. Although the study provided a valuable insight into the diet characteristics of parents and their infants, it recorded foods offered to infants but not necessarily consumed (or how much was consumed) and therefore there can be no inference on the nutritional adequacy in the diet of this group. Apart from the small sample size and the fact that the research was conducted via the internet and therefore open to bias (which are limitations acknowledged by the authors), intakes of nutrients from the parental diet records did not take into account the potential confounding effect of energy intake or any socioeconomic factors (which were not collected at the beginning of the study). Another recent study by Townsend and Pitchford looked at the effects of method of complementary feeding (baby-led versus spoon-fed) in a small sample of infants on food preferences and liking of specific foods and food groups. They found that the only difference in preferences between the baby-led and the spoon-fed groups was among carbohydrates (p=0.003) and that the baby-led group liked carbohydrates the most (although it is not clear if the term refers to starchy carbohydrate or total carbohydrate), as opposed to the spoon-fed group who liked sweet foods the most. There were however no quantitative data on actual intakes (Townsend & Pitchford 2012).

2.5 Timing of solids introduction and iron status

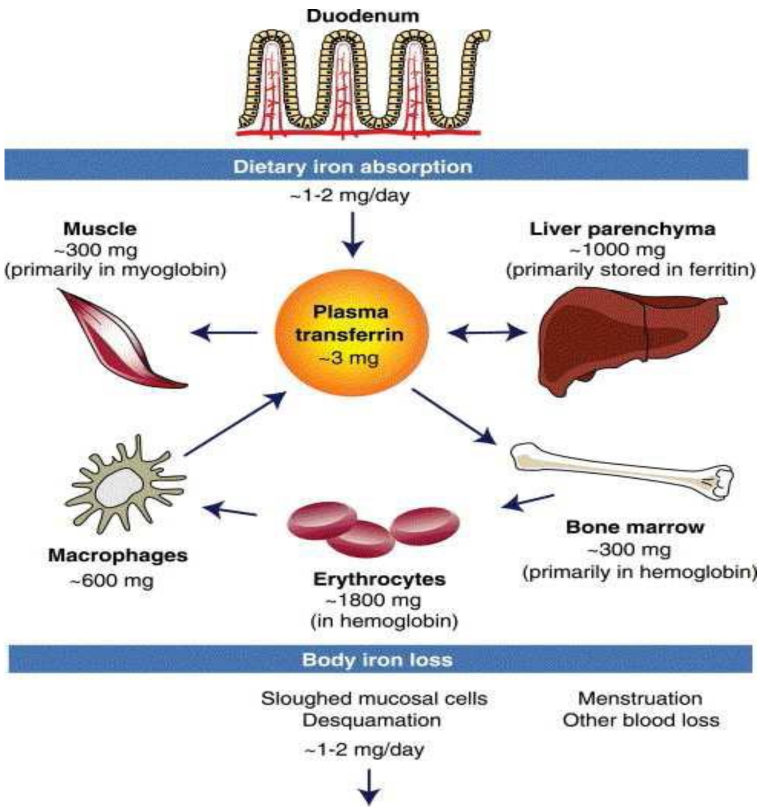
2.5.1 Iron function and distribution

Iron is an abundant trace element essential for almost all living organisms. Due to its ability to readily donate and accept electrons, it plays an important role as part of enzymes in many physiological processes, such as mitochondrial respiration, the citric acid cycle and DNA synthesis. It also plays an important role in oxygen transport as part of haemoglobin as well as in oxygen storage and use in muscles as component of myoglobin (Geissler & Singh 2011). However, its high reactivity could render it potentially toxic (Chua et al. 2007). Due to the highly hazardous nature of free iron, it is always bound to high affinity proteins in the human body. The human body contains about 3-5g of iron The distribution of iron in the body is shown in Figure 2-2 (Papanikolaou & Pantopoulos 2005). There are three pools of iron distribution in the human body (Edison et al. 2008):

- Functional iron as Haemoglobin and Myoglobin (Muñoz et al. 2009).
- Transport iron as Transferrin (Tf) (Muñoz et al. 2009).
- Storage iron as ferritin and haemosiderin (Scientific Advisory Committee on Nutrition 2010).

Iron is obtained solely from dietary sources (Nadadur et al. 2008). Dietary iron is found in two forms: haem iron (10% of total dietary iron intake in a typical western omnivorous diet) which derives from the haemoglobin and myoglobin of animal sources and non-haem iron (90% of total dietary iron intake) which derives from plant sources (Beard and Han 2009). The average western diet contains 15-20mg of iron per day, however only 1-2mg of iron is absorbed (Muñoz et al. 2009). This is because it is estimated that 15-35% of haem iron can be absorbed as opposed to less than 10% of inorganic non-haem iron (Zimmermann & Hurrell 2007).

Figure 2-2 Iron distribution



(Papanikolaou & Pantopoulos 2005)

2.5.2 Assessment of iron status

The spectrum of iron status extends from iron deficiency to iron sufficiency and excess. Assessment of iron status therefore involves determining where on this spectrum an individual's iron markers lie. As there is no one single marker for assessing iron status, interpretation of a combination of markers is needed in order to diagnose deficiency or excess. Even then, cut-off values are not necessarily diagnostic but the greater the deviation from the reference ranges, the higher the chance of the existence of a pathological iron status (Scientific Advisory Committee on Nutrition 2010). Iron deficiency can occur due to the interaction of three risk factors: increased requirements (e.g. pregnancy), limited supply (e.g. malnutrition) or excessive losses (e.g. haemorrhage) (Muñoz et al. 2009). There are three iron deficiency states:

- Iron deficiency (ID): Also referred to as depleted iron stores (Beard & Han 2009) whereby cellular storage iron in depleted with or without anaemia (Zimmermann 2008).

- Iron deficiency anaemia (IDA): is defined as iron deficiency and low haemoglobin. Clinical diagnostic criteria include low serum transferrin saturation (<15%), low serum ferritin concentration (<12µg/l) and elevated soluble TfR (≥6mg/l) (Beard & Han 2009).
- Iron deficient erythropoiesis (IDE): The circulating iron is reduced to such an extent that erythropoiesis is affected. It is often associated with inflammation and malignancy (Zimmermann 2008).

The World Health Organization reviewed all the markers for assessing iron status and concluded that haemoglobin is the best measure for assessing the severity of iron deficiency when used with other markers and that ferritin is the single most useful indicator of iron status unless an individual has widespread infection (in which case transferrin receptor concentration needs to be used additionally) (World Health Organization & Centers for Disease Control and Prevention 2007).

The WHO has defined anaemia in infants and children below 5 years of age as haemoglobin levels less than 110g/l and ferritin levels below 12µg/l (World Health Organization 2001). However, there has been concern that these values are too high especially for the first two years of life and overestimate the prevalence of anaemia in this age group. A study of breast-fed iron replete infants (as assessed at 4,6 and 9 months respectively) from Sweden and Honduras (n=263) suggested lower cut-offs for haemoglobin (105 g/l at 4-6 months and 100 g/l at 9 months) and ferritin (20µg/l at 4 months, 9 µg/l at 6 months and 5 µg/l at 9 months) (Domellof, Dewey, et al. 2002). In the UK, the Avon Longitudinal Study of Pregnancy And Childhood (ALSPAC) established cut-offs for haemoglobin and ferritin at the 5th centile of a randomly selected population. Haemoglobin levels were 97g/l at 8 months and 100g/l at 18 months (Emond et al. 1996) whilst ferritin levels were 16µg/l at 8 months and 12µg/l at 18 months of age (Sherriff et al. 1999).

2.5.3 Factors affecting iron status in the perinatal period

Foetal iron stores during gestation are estimated to contain about 250mg of iron (Zimmermann & Hurrell 2007). Approximately 60% of this is accumulated during the third trimester of gestation (Rao & Georgieff 2007). Iron requirements in pregnancy are increased in order to meet maternal blood cell expansion as well as foetal development

(Tapiero et al. 2001). However, there is no need for extra iron intakes in pregnant women as increased absorption of dietary iron, savings from cessation of period and mobilisation of cellular iron stores make up for the increased demand for iron(Committee on Medical Aspects of Food Policy (COMA) 1991). A ferritin level of 12 μg/l seems to be the cut-off point below which foetal iron accretion may be affected (Rao & Georgieff 2007). Therefore even mild to moderate maternal iron deficiency does not affect foetal iron acquisition (Rao & Georgieff 2002), although these infants are at a higher risk of developing iron deficiency anaemia between 6 and 12 months of age (Rao & Georgieff 2007). Severe maternal iron deficiency can affect foetal iron stores (Dewey & Chaparro 2007) and can affect 30-50% of pregnancies worldwide making it the most common perinatal cause of iron deficiency. Also, severe maternal copper deficiency can affect iron metabolism and can lead to anaemia and build-up of iron in the hepatocytes. The foetus is directly affected by receiving less iron and therefore has an increased risk of being born prematurely and/or with a low birth weight (Gambling et al. 2008). Perinatal iron deficiency can be caused due to gestational conditions of the mother such as diabetes mellitus, smoking and intrauterine growth restriction. They all cause through different mechanisms foetal hypoxia which in turn increases erythropoiesis and therefore increases foetal iron requirements (Rao & Georgieff 2007). Delayed cord clamping contributes to the increased neonate iron endowment. Chaparro and colleagues (Chaparro et al. 2006) found that a 2 minute delay in cord clamping increased iron stores at birth and at 6 months, even in low birth weight infants or infants born to iron deficient mothers. Prematurity and the associated low birth weight are also risk factors for iron deficiency. As the majority of iron stores is obtained during the third trimester, the preterm infant is deprived of that chance so premature infants have both lower haemoglobin and ferritin concentrations at birth compared to a full-term infant (Rao & Georgieff 2002). Moreover, catch-up growth and increased postnatal erythropoiesis cause the early depletion of the already compromised iron stores in premature infants. Combined with additional iatrogenic causes such as delayed iron supplementation, blood losses during medical procedures (such as phlebotomy) and recombinant erythropoietin practices without iron supplementation (Rao & Georgieff 2007), premature infants are at increased risks of iron deficiency states.

2.5.4 Infant iron metabolism in the first six months of life

After an initial period of physiological anaemia, haemoglobin levels start to increase from 2 months and there is a shift in iron distribution in the body although the total body iron (TBI) content stays stable up until 6 months of age. These changes in iron content and distribution from birth to 6-12 months are displayed in Table 2-2 (Dewey & Chaparro 2007).

Table 2-2 Changes in iron content and distribution in the first six months of life

Age (months)	Haemoglobin (% TBI)	Ferritin (%TBI)	Myoglobin and iron containing enzymes (%TBI)	TBI content
0	70	24	6	260 mg
4	76	12	12	~ 260mg
6	76	12	12	420mg[1]

TBI: Total Body Iron

[1]by 12 months

(Dewey & Chaparro, 2007)

Iron status in early infancy depends on its endowment at birth and rate of growth. Infants who gain weight rapidly have a higher risk for developing iron-deficiency anaemia (Dewey & Chaparro 2007). The iron content of breast milk is 0.4-0.8 mg/l in the colostrum (first milk) and 0.2.-0.4mg/l in mature milk. Due to the presence of homeostatic mechanisms of regulation in the mammalian gland (Butte et al. 2002), its iron content is not altered by maternal diet and iron status content (Krebs & Hambidge 2007). Breast-fed newborns receive iron in two forms: iron bound to lactoferrin and non-haem iron. Non-haem iron is loosely bound to molecules such as citrate and xanthine oxidase whereas two atoms of ferric iron are bound to the iron binding protein lactoferrin. There is evidence to support lactoferrin’s role in the higher bioavailability of iron in breast milk than the iron from formula milk (Sarkar 2004). At six months of age it is estimated that 16-25% of iron in breast milk is incorporated into the infants’ erythrocytes. Using an average absorption rate of 20% and with milk iron content at 0.3mg/l an infant consuming 800ml/day of breast milk will absorb around 0.05mg of iron per day. This would not be sufficient to meet iron needs unless iron is utilised from the infants’ reserves (Scientific Advisory Committee on Nutrition 2010).

2.5.5 Iron in the second six months of life

Due to the high rate of infant growth and development, iron requirements in the second 6 months of life are increased and milk (either formula or breast milk) alone is not capable of meeting those requirements. Crucially, there is a need for dietary iron intake from complementary foods even if breastfeeding is still continuing (Grant et al. 2007). Indeed, iron has been identified as a "problem nutrient" during the complementary feeding period (Brown 2007), as there is a gap between the iron intake from weaning foods and iron requirements of infants in this age group. Therefore the dietary composition as well as the timing of introducing solids will affect iron status in infants. An investigation of iron intakes in infants from the ALSPAC study (n=918) showed that at 8 months, the main contributors of iron in breast-fed infants' diet were commercial infant foods (consumed by 90.7% of infants) and breakfast cereals (consumed by 79.6%) followed by meat/fish (64.6%) and vegetables (97.6%). Iron intakes in breast-fed infants who received less than 6 breastfeeds per day were significantly higher than those infants who were breastfeeding more (≥ 6 feeds/day) (6.2 versus 5 mg/day, p=0.04) (Hopkins et al. 2007). However, in both, mean iron intakes were below the recommended nutrient intake for iron (7.8mg/day) as set by the Committee on Medical Aspects of Food and Nutrition Policy (COMA) 1991).

There are very few studies that have investigated the associations between timing of solids introduction and iron status in exclusively breast-fed infants. In the Honduras, Dewey and Cohen performed a randomised controlled trial to determine the effects of solids introduction in infants exclusively breast-fed until 4 months compared with infants exclusively breast-fed until 6 months. The study showed that infants who were introduced to solids earlier had significantly better haematological indices (haemoglobin, ferritin and haematocrit) by 6 months than the 6 month exclusively breast-fed group. However, the difference was small (around 4%) and a considerable proportion of the early complementary feeding group (20-25%) also had low haemoglobin and haematocrit levels. The authors concluded that early introduction of iron containing complementary foods may not be sufficient to prevent iron deficiency anaemia and that other factors, such as birth weight and umbilical cord clamping, should be taken into consideration. They also stressed the need for further research in both developing and affluent countries (Dewey et al. 1998).

Many of the complementary feeding guidelines stress the importance of including iron-rich foods in the complementary feeding diet. Krebs and Hambidge (Krebs & Hambidge 2007) suggest that meat needs to be introduced immediately after 6 months of age in order to provide iron, zinc and Vitamin B12 to the infant diet. The Paediatric Group of the British Dietetic association also recommends that high iron-containing foods including meat, oily fish and pulses should be introduced at around six months (British Dietetic Association 2013b). However, other sources of information (for example "Netmums" and the Start4life leaflet) and popular perception tend to suggest that meat should be introduced after the infant is well established on vegetables, which may delay the introduction of iron-rich foods in the diet, further compromising iron status.

Exclusively breast-fed infants, who did not have a good iron endowment at birth, continue to breastfeed exclusively after 6 months or are not introduced to iron-rich complementary foods at 6 months, are at a particular risk of iron deficiency.

2.5.6 Iron deficiency anaemia

Iron deficiency anaemia is a public health problem affecting both developed and developing countries and is considered to be one of the most important causes contributing to the world health burden (World Health Organization 2002). The World Health Organisation's report on the worldwide prevalence of anaemia between 1993-2005 (World Health Organization 2008) estimated that 8% (CI 5.9-10.8) of preschool children in the UK aged 1.5-4.6 years had haemoglobin levels below 110g/l, and characterised the prevalence as a mild public health problem. However, in the latest infant diet survey, 15% of infants aged over 12 months (n=325) had Hb levels below the reference cut-off of 110g/l, whereas 2% were classed as having iron deficiency anaemia, namely haemoglobin levels below 110g/l and ferritin levels below 12μg/l (Lennox et al. 2013). Consequences of iron deficiency include fatigue and cognitive and psychomotor development impairment (Scientific Advisory Committee on Nutrition 2010). Therefore, infants who are lacking this essential nutrient are at increased risk of neurological, growth and developmental problems which can persist to childhood and beyond (Lozoff et al. 2006). The symptoms of iron deficiency are usually mild and mostly go unnoticed. The deficiency is usually picked up after a random blood test or when the symptoms of fatigue are more severe. As the deficiency is so often undiagnosed, the American Academy of Pediatrics (AAP) in a recent report recommended universal screening for anaemia in toddlers at around one year (Baker et al. 2010). Although Hb is not thought to be a sensitive marker of iron deficiency or indeed iron deficiency anaemia, it was suggested that in the presence of low Hb levels, further testing of serum ferritin, transferrin, c-reactive protein and reticulocyte haemoglobin levels should be performed to confirm diagnosis (Baker et al. 2010). At the moment in the UK there is no universal screening for iron deficiency anaemia in place in primary care. Paediatricians are reluctant to perform extensive iron status testing in infants and young children due to the invasive nature of the venepuncture in order to obtain a blood sample. Taking blood samples from infants in the community to screen to iron deficiency anaemia would also present with several issues. For the health professional these include the cost, training and equipment required to obtain the blood samples in situations which may be difficult (such as home visits). For the infant (and the parent), the blood sampling process can be a painful and distressing experience and although it is classed as low-risk, there is the possibility of associated complications, such as infection and further blood loss from the wound.

A non-invasive technique could be a useful instrument to monitor iron status indices more frequently and earlier than blood testing, therefore resulting in faster detection and prevention of iron deficiency anaemia (Bamberg 2008). Such devices offer a promising alternative to conventional invasive blood sampling techniques. They have been successfully used to measure haemoglobin levels in preterm and term neonates with high correlation coefficients between the standard blood sampling methods and the non-invasive measurements (Rabe et al. 2005; Rabe et al. 2010). However, a non-invasive method of haemoglobin measurement has not been validated in free-living older infants.

2.6 Timing of solids introduction and eating behaviours

2.6.1 Development of food preferences

The complementary feeding period marks the beginning of a transition from a single food diet of milk (breast or formula) to a variety of semi-solids or solid foods of different textures and tastes. The first year of life is a sensitive period in acquiring taste preferences (Mennella et al. 2011); even though taste development is a continuing process, food preferences are formed at this very young age and track into childhood and adulthood (Coulthard et al. 2010; Nicklaus 2009; Kelder et al. 1994). Unhealthy food habits can therefore have not only a short-term, but also a long-term effect on health. It is now recognised that taste acceptance is a strong predictor of food consumption in children. Readily accepting new tastes will also lead to a greater variety of foods consumed. Dietary variety is a major element of a high quality diet which corresponds to adequate intake of essential nutrients, promoting good health and preventing non communicable chronic diseases (Ruel 2003). It is important therefore to understand the factors driving the acceptance of the very first foods, as these will form the basis of the child's future food choices. Several factors are known to be involved in the development of food preferences and they can be broadly categorised as genetic and environmental factors (Blissett & Fogel 2013).

Taste buds develop very early during gestation, with nerve cells forming on the tongue as early as the 6^{th}-7^{th} week of gestation (Witt & Reutter 1996). Newborn infants respond to different taste stimuli, as shown by facial expressions to ingested solutions of different tastants (Ganchrow et al. 1983). Infants react positively to sweet tastes by sucking eagerly, licking the upper lip and smiling. Sweet solutions seem to also have a calming effect on the infants (Ganchrow et al. 1983). Sour tastes induce a negative reaction in newborn infants characterised by opening the mouth, lowering corners of the mouth and nose wrinkling (Steiner et al. 2001). The effect seems to be reversed later in life with infants accepting sour tastes at 15-20 months or being indifferent to them. Bitter tastes are negatively received by newborns by lowering mouth corners, eye blinking and moving the head (Ganchrow et al. 1983; Steiner et al. 2001), although in some studies newborns have been indifferent to low concentrations of bitter tastes. Newborn infants seem to be indifferent or reject salty tastes during the first weeks and months of life, however, preference for salted water in observed in 6-24 month olds denoting a shift in the infant's response to salty tastes. It is worth noting that there are wide individual variations observed. In terms of umami taste, the limited research suggests that infants are indifferent to it from 3-12 months (Schwartz et al. 2011).

Everyone reacts differently to external sensory experiences depending on individual sensitivity to sensory stimuli. For example, children with hypersensitivities to other external stimuli such as sounds, smells and bright lights are also found to have sensory food aversions (Chatoor 2009) and therefore be more selective when it comes to trying new flavours, tastes and textures. Dunn proposed that there are certain neurological thresholds over which the nervous system reacts to external sensory stimuli (Dunn 1997). For example, a child with oral sensory sensitivity is more likely to show preferences for certain tastes or smells and avoid tastes that are a typical part of children's diets, be picky with regards to textures and seek out certain smells/tastes (Dunn 1997). Humans are also programmed to reject novel foods (neophobia). Unwillingness to try new foods peaks at the age of 18 months and lasts up to 7 years (Dovey et al. 2008). This ability has an evolutionary benefit: It coincides with the infants increased independence from parental supervision and is a protective mechanism against ingesting unknown and potentially harmful new foods (Pliner & Salvy 2006). Although there is evidence that neophobia has a genetic element and is heritable, it can also be related to parental modelling. For example, if parents are neophobic they are more likely to offer a more limited variety of foods to their offspring (Blissett & Fogel 2013).

Finally, infant temperament seems to be weakly but consistently related with neophobia; exposure to novel food could be construed like any other exposure to a new, unfamiliar experience and therefore is likely to incur similar reactions. Emotionality and shyness are related to higher incidence of food refusal and negative experiences during mealtimes (Pliner & Loewen 1997). Although genetic predisposition can explain part of the differences in taste learning and acceptance, these factors interact and can be influenced by a number of environmental factors.

The first exposure to outside flavours occurs in utero through the transfer of volatile compounds from the maternal diet to the foetus through the amniotic fluid. During the final months of gestation the foetus ingests large amounts of amniotic fluid, and with it, its components of nutrients and other compounds from the maternal diet. Indirect studies of foetal reactions to various tastants have shown that a sweet solution injected in the amniotic fluid resulted in increased foetal swallowing whereas a bitter solution resulted in the slowing of swallowing. This suggests that the infant responds differently to different taste exposures via the amniotic fluid in the later part of gestation (Ventura & Worobey 2013). The exposure of the infant to flavours derived from the maternal diet continues soon after birth with breastfeeding creating a “flavour bridge” or a “chemical continuity” between life in utero and birth. The flavour of breast milk is not inert but varies depending on the maternal diet. Breast-fed infants are more willing to try new foods especially those which are exposed to during breastfeeding (Forestell & Mennella 2007). This chemosensory learning is thought to help make a smoother transition to the next step of feeding, the introduction of solids. Breastfeeding therefore seems to facilitate later acceptance of the first complementary foods as they resemble tastes that they have already been exposed to (Cooke & Fildes 2011). However even in formula-fed infants there have been indications of learned food preferences. For example, infants who were exposed to a protein hydrolysate formula (which has a bitter taste) before 3.5 months were more likely to accept the same formula at 7.5 months compared to their counterparts who were exposed to it after 3.5 months or exposed to a cow’s milk formula instead (Mennella et al.

2011), indicating a potential early critical period in flavour learning. Therefore exposure to different tastes during milk feeding could lead to increased later food acceptance but the biggest effect is observed in breast-fed rather than formula-fed infants as they are exposed to a greater variety of flavours through breast milk (Cooke & Fildes 2011).

The next transition to the flavour learning process is the introduction of solids, where the infant is directly exposed to new flavours and textures through family foods. Most studies focus of the timing of cessation of breastfeeding rather than the timing of introduction of solids. Although the two are directly correlated, little is known as to whether the age of solids introduction independently affects flavour learning and subsequent food acceptance (Nicklaus 2011). Also, most studies focus on introduction of a variety of tastes but texture is also an important factor in later food acceptance. The following sections will assess the effect of age of solids introduction in both texture and taste acceptance and their consequences in feeding difficulties and dietary variety.

2.6.2 Food sensory properties and feeding difficulties

Learning to process different textures during the complementary feeding period is a challenging process, but a vital step in their acceptance. Some studies have hypothesised the existence of a sensitive period in the months following complementary feeding in which when new flavours and textures are introduced, they are more readily accepted (Harris 2008). Exposure of a variety of textures has been associated with higher food acceptance and less food refusals at 6 and 15 months (Northstone et al. 2001). Moreover, introducing foods with a lumpier texture at 10 months has been associated with higher rates of food refusal and a limited variety of fruit and vegetable consumption (Coulthard et al. 2009). In a study of 12 month old infants, acceptance of chopped carrots was associated with prior exposure and familiarity with a variety of textures in the months earlier (Blossfeld et al. 2007). Even though the majority of infants preferred the purred texture, those who were exposed to the chopped textures previously, were more likely to consume a greater variety of foods and be willing to eat more of both textures of carrots. Earlier introduction of solids was also a predictor of texture acceptance, however there was no information as to when the infants were introduced to puree or chopped textures. Another interesting finding with regards to acceptance of different food textures is the fact even though early consumption of raw and home cooked vegetables predicted their consumption at 7 years, the same was not observed for commercially prepared fruit and vegetables

(Coulthard et al. 2010). This effect was also observed by Birch who suggested that the texture differences between the two types of foods may be responsible for this (Birch et al. 1998).

2.6.3 Food preferences and dietary variety

Children's food preferences play an important role in future food choices and are associated with consumption (Birch 1999). The WHO recommends a varied diet when complementary feeding begins (World Health Organisation 2002) and throughout infancy and childhood. Infants' consumption of one food could enhance acceptance of similar foods (Birch et al. 1998) and therefore taste acceptance can influence dietary variety. Most studies looking at acceptance of foods during the complementary feeding period have focused on fruit and vegetables as they are of public health importance for long-term health and disease prevention, they are characterised by less desirable tastes (bitterness for a lot of vegetables and sourness for some fruits) and they are some of the first solids foods usually introduced to infants. Vegetables in particular are characterised by bitter tastes and are less easily accepted by infants compared to the sweet taste of fruits (Carruth et al. 2004).

Exposure to a variety of these foods in early weaning seems to be an important factor in food acceptance. A 12-day experiment by Gerrish and Mennella assessing the role of variety in acceptance of novel flavours involved 48 exclusively formula-fed infants. Mean age of first solids introduction (cereals) was 4±0.1 months which meant that at the start of the experiment infants had already been having cereal for 3.8±0.4 weeks. Half of the sample had also consumed fruit or fruit juices before the experiment either daily or occasionally. The infants were separated in 3 groups; a group fed pureed carrot from 10 days, a group fed pureed carrot on day 1 and mashed potato for 9 days and a group that was fed carrot on day 1 and a different pureed vegetable for 9 days. At day 11, all 3 groups were fed pureed carrot and at day 12 pureed chicken. Infants in the variety group accepted the novel taste of chicken easier and ingested more of it than the other two groups, indicating that a variety early in the weaning period enhances the acceptance of other novel tastes (Gerrish & Mennella 2001). A study of a similar design which included both breast-fed and formula-fed infants showed that the combination of breastfeeding and food variety early in the weaning process combined produced a stronger effect of acceptance of a novel flavour (Maier et al. 2008). Although the above studies highlighted that variety early in the complementary process is beneficial for later novel food acceptance, they did not directly assess the effect of different time points of solids introduction in the acceptance of novel flavours and therefore the consumption of a variety of foods.

Howard and colleagues in Australia (Howard et al. 2012) looked at maternal and child predictors of vegetable, fruit and non-core foods (foods high in fat or sugar) preferences in 245 2-year old infants. Maternal food preferences were significant predictors of fruit, vegetables and non-core (foods high in sugar, fat or salt) food liking; neophobia also significantly predicted liking of fruit and vegetables. However, age at which solids were introduced did not predict fruit and vegetable liking but it predicted non-core food liking, with infants introduced to solids earlier liking more non-core foods, after adjustment for maternal socioeconomic factors, child gender and birth weight. Recall bias could have been a potential factor in this study as mothers were asked about their infants age of solids (in weeks) when they were 13 months old. Also food neophobia which peaks at that age could have overpowered other predictors. Early exposure seems to be the most effective method of flavour learning and food acceptance. In a study with 12 breast-fed infants, salty cereals were more accepted between 16-17 weeks rather than 18-25 weeks, indicating an early window of acceptance of salty tastes (Harris et al. 1990). However, just like any other sensory learning there may be more than one window of plasticity in flavour learning (Mennella et al. 2011). Similarly, in a study of four European cohorts (ALSPAC in the UK, EDEN in France, Generation XXI in Portugal and Euro-Prevall in Greece), the age of introduction of fruit and vegetable intake was not associated with higher fruit and vegetable intake later in life in 3 out 4 cohorts. In ALSPAC, lower age of introduction of vegetables resulted in higher vegetable intake at 13 years of age, whereas lower age of fruit introduction resulted at higher intakes at 2 years of age, but not later (de Lauzon-Guillain et al. 2013). ALSPAC was the oldest of the 4 cohorts and therefore infant feeding recommendations for solids introduction were different than more recent studies; the largest proportion of infants were introduced to solids between 3-4 months in ALSPAC, compared to around 4-5 months in the Generation XXI study and around 5-6 months in the Euro-Prevall cohort. In EDEN, the distribution of age of solids introduction was fairly equal between 3-6 months. It could be therefore argued that very early age of solids introduction (less than 4 months) may be a significant factor in fruit and vegetable acceptance but later introduction of solids (after 4 months) may not be.

Other studies have also shown that despite the initial advantage of breastfeeding, formula-fed infants who are exposed to a wider variety of tastes during the period of complementary feeding are subsequently more accepting of novel foods compared to the beginning of the complementary feeding period (Forestell & Mennella 2007; Gerrish & Mennella 2001).

From these studies, it can be assumed that there are sensitive periods in taste acceptance during complementary feeding and that breastfeeding confers an advantage to accepting new favours compared to formula feeding. It is also evident that that parental experiences with food are also important in shaping infants' preferences (Harris 2008). Most of the evidence however seems to be focusing on the consequences of breastfeeding cessation and there is inconsistency when it comes to the effect of timing of introduction of new foods during flavour learning. Early and repeated exposure has been shown to be effective in fruit and vegetables acceptance, however how is early defined? The evidence is clear about the detrimental effects of introduction of solids before 4 months but there is still confusion regarding the 4-6month window and the 6 month time point, currently set by the Department of Health in their infant feeding guidelines. Not many studies have compared the two cut-off points and yet we know from the latest infant feeding survey that more and more mothers, irrespective of breastfeeding exclusively, are waiting until 6 months to introduce solids (McAndrew et al. 2012b). As noted before, this would require a faster transition period through flavours and textures so how is this going to affect the acceptance of new foods?

Although it is well established that the sensory properties of foods play an important role in acceptance, there is a lack of studies looking at foods other than fruit and vegetables such as meat, which is also an essential part of the infants' early diet as it provides a source of bioavailable iron as discussed previously. Are infants who are introduced to these foods at 6 months less receptive to these flavours than those exposed to them before 6 months? Does that affect the acceptance of other foods and impact on dietary variety? These are questions that need to be addressed in order to provide parents useful advice during this challenging period of feeding transition (Nicklaus 2011).

2.7 Chapter summary

The review of the literature has shown contradicting results in studies looking at anthropometric, dietary, haematological and behavioural outcomes and age of solids introduction in infants. Very few studies have used 6 months as the cut-off point for early and late introduction of solids and no such studies have been conducted recently in the UK. The relatively new practice of baby-led weaning also warrants further investigation into its association with the outcomes mentioned above, in order to be able to establish its role in complementary feeding practices of infants. Even though iron deficiency anaemia has been recognised as a public health issue, the invasive nature of testing for iron indices may delay the diagnosis and treatment of anaemia in infants and toddlers. These areas would therefore benefit from further investigation.

In the Brighton's Breast-fed Babies Study (Chapter 3), growth, diet, iron status and eating behaviours will be explored in principally breast-fed infants stratified by age of solids introduction (before and after 6 months) and by mode of complementary feeding (baby-led weaning and spoon-feeding).

Moreover, a non-invasive device in measuring iron status will be validated against a standard blood sampling technique in the Haemospect® Validation Study (Chapter 4). Finally, in the GO-CHILD Study (Chapter 5), food preferences dietary variety and other eating behaviours will be examined in mixed-fed infants stratified by age of solids introduction (before and after 6 months). Specific aims and objectives for each study will be described in each of the individual chapters.

Chapter 3

The Brighton's Breast-fed Babies Study

3 The Brighton's Breast-fed Babies Study

3.1 Chapter overview

This chapter will describe anthropometric, diet, haematological and behavioural aspects in in a group of infants from Brighton and Hove who followed closely the current infant feeding recommendations, by examining them in 2 different ways: by age at which they were introduced to solids (before and after 6 months) and by mode of complementary feeding (baby-led weaning and spoon-feeding). The results will then be discussed and placed within the context of current literature.

Aims, Objectives and research hypotheses

The Brighton's Breast-fed Babies Study aimed to describe the growth, diet, iron status and feeding behaviours of principally breast-fed infants in Brighton and Hove at 8 and 18 months. The infants were examined initially as a whole and then stratified in 2 different ways: by age at which they were introduced to solids as well as by mode of complementary feeding (baby-led weaning or spoon-feeding).

Aim 1: To compare growth patterns, food and nutrient intakes, iron status and eating behaviours at 8 and 18 months between principally breast-fed infants from Brighton and Hove who were introduced to solids before and those introduced to solids after 6 months.

Aim 2: To compare growth patterns, food and nutrient intakes, iron status and eating behaviours at 8 and 18 months between principally breast-fed infants from Brighton and Hove who followed baby-led weaning and those who were spoon-fed.

Objectives:

- To compare growth patterns of principally breast-fed infants with the WHO growth standards across the whole sample and between groups
- To assess nutritional adequacy of the weaning diet by comparison with the dietary reference values across the whole sample and between groups
- To measure haemoglobin levels and compare them against current cut-off values for anaemia across the whole sample and between groups
- To identify the presence of parent-reported feeding difficulties and other eating behaviours across the whole sample and compare differences between groups

Hypotheses to be tested

There are no differences in growth patterns, food and nutrient intakes, iron status and eating behaviours at 8 and 18 months between principally breast-fed infants from Brighton and Hove who were introduced to solids before and those introduced to solids after 6 months.

There are no differences in growth patterns, food and nutrient intakes, iron status and eating behaviours at 8 and 18 months between principally breast-fed infants from Brighton and Hove who followed baby-led weaning and those who were spoon-fed.

3.2 Methods

3.2.1 Study Design

The Brighton's Breast-fed Babies study was an observational cohort study. It consisted of an initial telephone/email contact and three home visits, at 6, 8 and 18 months.

3.2.2 Settings

Mothers of breast-fed infants were recruited from October 2009 until April 2010. They were recruited in the Brighton and Hove area although two of the participants resided in neighbouring towns. Recruitment was conducted via posters in GP surgeries and pharmacies, local companies, advertisements in the ABC magazine (a free local parenting magazine) and through visits to breastfeeding support groups and baby clinics.
Data collection was performed in the participants' homes from November 2009 until May 2011 (last food record collected in July 2011). Participants were followed up for 12 months in total.

3.2.3 Participants

The inclusion criteria for the selection of participants were full-term singleton infants (37-42 weeks gestation), aged less than 6 months with mothers breastfeeding (exclusively or partially) at 4-5 months and intended to continue until the baby was at least 6 months old. Only infants from singleton births were included, as twins and other multiples are likely to differ from singletons in terms of iron status at birth (Luke 2005). Preterm births were also excluded, as this was likely to affect both iron status and when the parents decided to introduce solids foods (Siddappa et al. 2007) (Rao & Georgieff 2007). A local address (recruitment within Brighton and Hove) and the ability of the mother to speak and read English were also required, as there were insufficient funds to supply translators and interpreters.

During the recruitment stage and to avoid confusion to the participants with the term "partial breastfeeding" all mothers who were using any amount of breast milk were included. During the initial telephone/email contact, mothers were asked how many of their infant's feeds were breast milk and how many were formula within one day. Mothers feeding their infants with at least four out of five feeds breast milk [80% breast milk, considered to be high breastfeeding intensity as defined by Labbok et al. (Labbok & Krasovec 1990) then continued to be part of the study].

Infants who did not fall into the above inclusion criteria, those with major congenital abnormalities, congenital anaemias, diagnosis of anaemia, prescribed iron supplementation, receiving sulphonamides (the use of sulphonamides may have harmful effects on red blood cell production and cause anaemia) or diagnosed with any chronic conditions likely to affect food intake or absorption were excluded from the study.

The inclusion and exclusion criteria were operationalised by asking the mother about the baby at the initial telephone/email contact. The ability to speak and write in English was also assessed at this stage by the researcher.

3.2.4 Data collection

3.2.4.1 Data collection timeline

When the child was around 4-5 months old, prospective participants who had initially expressed interest in the study were contacted by telephone or email. Participants were asked if they were still willing to take part in the study and planned to breastfeed their child (fully or partially) until he/she was at least 6 months old. If so, the researcher sent further information in the post and arranged to visit mothers in their home when their infants were aged around 6 months.

6 month visit

At this visit the researcher discussed any queries about the informed consent for participation in the study and obtained it. Three separate consent forms were given: one for participation in the questionnaire and dietary data collection (just before the 6 month visit) and two for taking capillary blood samples from the infant (just before the 8 month and 18 month visit respectively). It was made clear to the mothers that they were free to withdraw from the study or withdraw permission for blood taking at any point and without having to give a reason. At this stage the blood taking process was explained and a blood sampling consent form was provided. This was left with the mother to complete when the child was aged 8 months. A questionnaire collecting background information was also collected during this visit. For assessment of the weaning diet, a three day food record was provided along with detailed instructions on how to complete it. This was left with the mother to complete when the child was aged 8 months. Finally, baseline measurements of weight and length were taken from the infants' Red Book and a second visit at around 8-9 months of age was arranged.

8 month visit

At this visit, the researcher discussed any queries about the informed consent for blood sampling and obtained it. If the food record and accompanying questionnaire had been completed, they were discussed with the mother in order to make sure that there were no anomalies and that the information provided was clear and complete. If consent was granted, a capillary blood sample was collected from the infant for the haemoglobin measurement. The infant's weight and length were also measured and recorded. Any recent weight and length measurements available in the infant's "Red Book" were also recorded. Finally, a blood sampling consent form as well as another three-day food record with an accompanying food questionnaire were provided, and mothers were advised to complete these when the child was 18 months old.

18 month visit

The 18month visit was similar to the 8 month visit; weight and length were measured and recorded and if consent was granted, a heel prick blood sample was obtained for haemoglobin measurement. Any recent weight and length measurements from the infants'

Red Book were also recorded. The researcher went through the food record with the mothers and clarified any discrepancies or missing information.

3.2.5 Variables

3.2.5.1 Background questionnaire

Parental characteristics in the background questionnaire included: maternal age (measured in years), ethnic group (dichotomised as "white" or "other"), maternal education (categorised as highest qualification: "postgraduate qualification", "degree"," A levels" or "no qualifications"), housing tenure (categorised as "owned/mortgaged", "owned/no mortgage to pay" and "rented"), smoking (categorised as "current smoker", "former smoker" or "never smoked") and iron supplementation during pregnancy ("yes", "no" and a further category "yes but did not take it"). Infant characteristics included birth weight and gender. A copy of this questionnaire can be found in Appendix 1.

3.2.5.2 Growth measurements

Weight measurements were taken at 8 and 18 months at the participants' homes, using high specification portable calibrated infant scales Seca 384 (III) in accordance with Precision Class III of EC Directive 2009/23/EEC, approved for medical use. The scales were used exclusively for the purpose of the study. The research student was trained by research nurses from the Cambridge Baby Growth Study. Standardised procedures were followed for each measurement. The weighing scales were placed on a level, fixed surface. The scales were then zeroed and cleaned, using an infant wipe. A paper sheet was put on the surface. The parent placed the infant on the scales. Infants and toddlers were weighed naked, without a nappy. The infant stayed still for a few seconds until the measurement was taken and recorded. Toddlers who were unsettled or distracted were held by the parent and both weighed together. The parent's weight was then taken separately and subtracted from the total weight. Alternatively the parent sat on the scales, which were zeroed. The parent, while still on the scales, held the child and both were weighed together, so the scales showed the weight of the child only. Measurements were made to the nearest 0.1kg. The scales were then cleaned as per instruction manual. The measurements were transferred from the data collection sheet to a locked and password protected computer database for analysis.

Supine length was measured at 8 and 18 months using the Seca 210 infant/toddler measuring mat. Because the infants/toddlers were aged below 2 years for both visits, length was measured lying down. The measuring mat was foldable with a measuring range of 10-99cm.The length of the toddler was measured without any clothing, including the nappy, which can distort the hips and shorten the length measurement. The mat was wiped clean. A paper sheet was then placed on the surface. The parent placed the infant on the mat. Two people were needed to obtain an accurate length measurement. The toddler lay on his or her back with one person (the parent) holding the head against the headboard with both hands. The corner of the toddler's eye was in a vertical line with the middle of the ear (known as the Frankfort Plane). The researcher gently flattened the knees, flexed the ankles of the toddler to 90 degrees and brought the footboard up to the flat soles of the flexed feet. Length measurement was taken to the nearest mm. The first length measurement was then repeated. If it was within 0.5cm of the first reading it was accepted, if not it was repeated again. The mat then cleaned as per instruction manual. The measurements were transferred from the data collection sheet to a locked and password protected computer database for analysis.

3.2.5.3 Dietary assessment

A detailed account of dietary assessment methods is beyond the scope of this thesis and has been extensively reviewed elsewhere (Thompson & Byers 1994). Although the golden standard of dietary assessment is the 7 day weighed food diary (Bingham et al. 1994), when assessing dietary intakes in young infants and toddlers fewer days are required as there is less variability in their diet (Lanigan et al. 2004). Diet diaries compare well with 7 day records, are more detailed than food frequency questionnaires, are less prone to recall bias and have a satisfactory test retest reliability (Eck et al. 1996). They have also been used successfully in assessing diets of infants and toddlers in the ALSPAC study (Emmett 2009). Therefore they were thought to be an appropriate method of dietary assessment in this study to balance providing sufficient detail of intakes whilst minimising participant burden and to ensure comparability with ALSPAC as the same time points are used in dietary assessment in both. Diet was assessed by 3 day un-weighed food diary, comprising of 2 weekdays and one weekend day at 8 and 18 months. At the 6 month home visit, the 8 month food diaries were given to the participants along with instructions, a portion size guide and a crib sheet with examples of how to complete it. The researcher went through the diary and explained how to fill in each section. Mothers were encouraged to contact the researcher if they had any queries. The 18 month food diary was posted 2 weeks before the 18 month visit along with the same portion size guide as in 8 months and an updated crib sheet with age appropriate examples of description of foods.

Parents were asked to record all foods and drinks that the infant had consumed for 3 days both at home and outside, using household measures and providing as much information as possible. Food intake assessment included time and place the food was consumed, a full description of the food item (to include cooking methods, portion size, brand, any other additions such as butter) whereas drink intake assessment included type of drink, dilution ratio (if applicable), amount consumed and any other additions (such as sugar). Separate sections were given in the diary to record foods, milk feeds and other drinks. They were also asked to record any vitamin supplements or medicines that their infant received during the recording day. A comments section was also available for the parent to describe any other relevant information. The crib sheet provided at each visit gave examples of portion sizes using household measures and how to extract information from packaging. An additional questionnaire was used to record recurrent foods or usual types of staple foods (such as type of butter, bread or milk usually used) in order to avoid repetition of details in the diaries and thus reduce the burden on the participants. This information was used to provide more details when coding. A copy of the short questionnaire used is given in Appendix 1. Participants were asked to record the amount of food consumed rather than what was initially offered. They were asked to record brand names where possible and keep food labels of unusual foods. For homemade dishes, parents were asked to record the recipe (ingredients, cooking methods) for the whole dish and the proportion of the finished dish that the infant consumed.

For each day they were asked if it was a typical day for the infants, if they have been unwell, if anyone else had looked after the child during mealtimes and if they had offered them any food or drink during that time. The main diary was divided into mealtime sections (before breakfast, breakfast, mid-morning, lunch, tea, evening meal, late evening and through the night). At the end of the diary there was a separate section for recording drinks but participants were also given the option to record drinks in the main section of the diary if they found it easier.

The diaries were intended to be checked during each home visit; however that was not always possible due to distraction from the infant. In some cases mothers had not had the chance to complete the food diary prior to the home visits and therefore the diaries were returned by post at a later stage. If there were any queries, the mother was then contacted by phone or email to provide clarifications.

The completed diaries were checked by the researcher and data was coded at the Human Nutrition Research (HNR) Unit in Cambridge using their in-house dietary coding system DINO (Diet In Nutrients Out). DINO is a Microsoft access database which incorporates dietary coding and nutrition analysis using the MacCance and Widdowson's food composition tables and its supplements. The database is updated constantly with new food items that become commercially available.

Each food item from the diaries was matched as closely as possible to a food code from DINO. When there was not sufficient information for a specific food, an alternative food was used instead and the decision was recorded to ensure consistency with future entries. DINO has an in built portion size descriptor based on household measures corresponding to weights, which is adapted from Food Standards Agency Food Portion Sizes (Food Standards Agency 2005) and manufacturers' information. When a portion size was missing, if the food had been consumed before (either on the same day or the other 2 days) that portion was used; if there was no other mention of the same food in any of the diaries, an average portion for the age group was used instead from the Food Portion Sizes book (Food Standards Agency 2005). For commercial foods and drinks not included in DINO, information on ingredients was collected via the food label if it was included in the food diary or the manufacturer's website. Each ingredient and its proportion were entered manually in DINO as individual food items. These items were then grouped in a recipe food group to account for the fact that they were consumed as a single food item. Uncooked foods such as salads and sandwiches were coded as separate food items and were not grouped together as a recipe.

For homemade dishes where a recipe had been recorded in the food diary, the ingredients were entered individually using the appropriate cooked food codes, and all the codes for the dish were allocated to a recipe food group according to the type of dish. DINO automatically calculated the weight of each ingredient by using the raw ingredient weights (from the recipe in the food diary), a weight loss or weight gain factor for the whole dish (from a comparable recipe in McCance and Widdowson's The Composition of Foods series) and the weight of the portion consumed. When a recipe was not included in the food diary, a standard homemade recipe food code was used.

Breast milk intake was assessed by volume of milk per minute of feed. This was based on data from a study by Paul and colleagues (Paul et al. 1988) who calculated breast milk intakes in infants aged 2-10 months. At both 8 and 18 months of age breast milk intakes were estimated as 10g/minute up to a maximum of 100g per feed, to account for comfort feeding.

3.2.5.3.1 Food Groups

Each food code from the food diaries was automatically allocated to subsidiary food groups in DINO (2 levels) which corresponded to 30 food groups. The food groups were then aggregated to 18 main food types. Some food types did not have any consumers (supplements, nutrition powder and drinks) and therefore were not included below. The food types were:

- Cereals and cereal products
- Milk and dairy products (excluding breast milk)
- Meat and meat products
- Fish and fish dishes
- Eggs and egg dishes
- Vegetables and potatoes
- Lentils and pulses
- Fruit
- Nuts
- Savoury snacks
- Infant Formula
- Breast milk
- Commercial baby foods

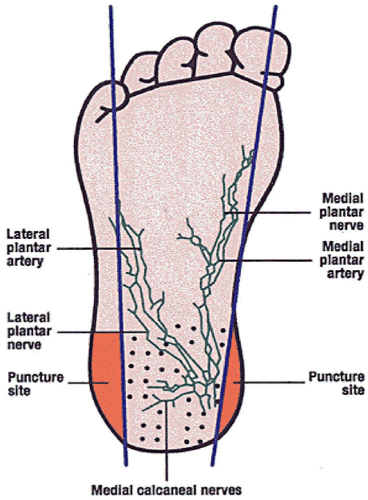
3.2.5.3.2 Nutrient analysis

DINO's nutrient databank was used to calculate nutrient intakes for each food item entered in the database. It is based on McCance and Widdowson's Composition of Foods series and manufacturers' data. All nutrients were found to be significantly correlated to total energy intakes, meaning that infants who consume more energy would also consume on average more of each individual nutrient. It was therefore essential to control each nutrient for the effect of total energy intake. To achieve this, the nutrient residual (energy adjusted) model was used as described by Willett and colleagues (Willett et al. 1997). Each nutrient (dependent variable) was regressed against total energy intake (independent variable) in a simple linear regression, producing unstandardized residuals. The residuals have a mean value of zero (because they include negative values) and cannot be interpreted on nutritional terms. Therefore a constant was added to each residual in order to resemble an actual nutrient intake. The constant used was the expected mean nutrient intake which also derived from the regression model.

3.2.5.4 Blood sampling

Haemoglobin was measured in heel-prick blood samples collected at 8 and 18 months using HemoCue® Hb 201+ (Ängelholm, Sweden) at the participants' homes. The lancing device used was Tenderfoot newborn (1mmx 2.5mm) blue/pink. The measurement was taken in a warm room and the infant wore socks to keep the heel warm prior to the procedure. The infant was placed in a secure position, cuddled by the parent. The parent was asked to remove the socks and using gloves the researcher cleaned the area with an antiseptic swab and waited until it dried.

Figure 3-1 Appropriate puncture sites on the infant's heel for the heel prick measurement of haemoglobin levels



The device was removed from its packaging and the safety clip was removed. A safe incision site was selected. The site was marked either by an imaginary line extending posteriorly from a point between the 4th and 5th toes and running parallel to the lateral aspect of the heel, or by a line extending posteriorly from the middle of the great toe running parallel to the medial aspect of the heel. The heel prick was performed on the planter surface of the heel beyond the lateral and medial limits of the calcaneus (Figure 3-1).

Before the device was activated it was placed firmly against the skin. The centre point of the device was vertically aligned with the incision site and both ends of it made light contact with the skin. The trigger was then pressed and the device removed. A micro-cuvette captured a single drop of blood from the incision point and after being wiped of any excess blood, it was inserted in the HemoCue® device. The measurement was then recorded after 60 seconds as per the device manual.

3.2.5.5 Eating behaviours questionnaire

Mothers were asked to fill in a supplementary questionnaire at 8 and 18 months along with the 3 day un-weighed food record. This questionnaire, based on those used at similar ages in ALSPAC, included questions about the types of foods they usually offer to their infant, feeding practices, preferences and feeding difficulties. More specifically mothers were asked to report:

- Timing of stopping breastfeeding
- Timing of introduction of foods with a lumpy texture and finger foods
- Perceived fussy eating
- Difficulties in feeding solids
- Parental perception of the infant’s feeding difficulties - under/over eating, food refusal, pickiness, problems with establishing an eating routine.
- Food avoidance- poultry ,fish, beef, other red meat, eggs, cheese, milk, butter, nuts, wheat/gluten, sugar
- Whether the infant ate differently than their peers
- Whether the infant was on a special diet

3.2.6 Data handling

All questionnaires, dietary records and blood samples collected were labelled with an identifier number and not the mother's or infant's name. The mother was informed of the probability of breach of confidentiality if there were indications of possible harm to her (including self-harm or threat by another member of the family) or others. As a token of thanks for their time in the study, mothers and infants received a children’s book at the last home visit. All relevant documentation for the Brighton’s Breast-fed Babies Study can be found in Appendix 1.

3.2.7 Bias

As the study required participants with breastfeeding intensity of over 80%, it was accepted that there was a strong likelihood of selection bias in the study population. In order to minimise that, every effort was made to promote the study in all parts of the city and especially in the most deprived areas. Moreover, the scope of the study was to examine the characteristics of breast-fed infants and therefore the selected sample was not meant to be representative of the whole population. Finally due to the study design there was no information available from people who decided against taking part in the study (as they did not have to make contact with the researcher) and therefore it was not possible to assess their characteristics and reasons for no participation (non-response bias).

3.2.8 Study size

Initial power calculations for the study were based on being able to detect a difference in iron status and nutrient intakes between children introduced to solids before and after 6 months.

3.2.8.1 Power calculation to detect a difference in iron status

The primary analysis initially involved testing for a difference between the two groups (introduced to solids before 6 months and after 6 months) in ferritin levels. In Emond and colleagues (Emond et al. 1996), ferritin was found to be log normally distributed and therefore the log transformed values were used in an independent sample t-test. The standard deviation of log ferritin for 8 month old infants was found to be 0.231. In a study investigating the effect of age of complementary feeding and iron status in breast-fed infants in the Honduras, Dewey and colleagues (Dewey et al. 1998) found a proportional difference of 1.42:1 in mean ferritin values between the two groups (67.1µg/l in the solids and breastfeeding versus 47.1µg/l in the exclusively breastfeeding group). Using these values and a 0.05 2-sided significance level to test the difference between the two groups, a sample size of 39 in each group would give 80% power to detect a difference of 0.15 on the log scale which would correspond to the geometric means being in the proportion 1.42:1.

3.2.8.2 Power calculation to detect a difference in nutrient intakes

Using vitamin C as one of the dietary outcome measures; at 18 months in the ALSPAC data in girls mean vitamin C was 48mg (Cowin et al 2000) and the standard deviation was 43mg. In order to detect a difference of 20mg in Vitamin C intake (with 80% power and alpha value of 0.05) 74 participants would be needed in each group.

A sample size of 150 would be sufficient to measure the dietary outcomes; additionally even with quite a high refusal rate for blood samples, there would be enough data to detect a difference in ferritin levels.

The aim was to recruit 180 participants, ideally 90 infants introduced to solids before 6 months, and 90 infants introduced to solids at or after 6 months. Allowing for dropout this would leave 75 infants in each group by the end of the study.

3.2.9 Statistical methods

The statistical software used for all analysis was PASW Statistics 21. All variables were explored for normality (using the Shapiro-Wilk normality test as the sample size was below 50). Results were presented either as frequencies (if the variables were categorical), means and standard deviations (SD) (if the variables were continuous and normally distributed) or medians and interquartile range (IQR: 25th, 75th centile) (if the variables were continuous and not normally distributed) across the whole sample and stratified by timing of solids introduction (before and at/after 6 months) and mode of complementary feeding (BLW and spoon-fed).

Because the age of solids introduction was not normally distributed, associations between age of solids introduction and anthropometric measurements at 8 and 18 months were investigated using Spearman's correlation. In order to be able to make comparisons with the WHO growth standards, all anthropometric measurements were entered into the WHO Anthro software (World Health Organisation, 2005) which compares growth data to the WHO Child Growth Standards by calculating z-scores. Weight-for-age z-scores were calculated for birth, 6, 8 and 18 months. For the 8 and 18 month time points, weight-for-length, length-for-age and BMI-for-age were also calculated. Independent sample t-tests (for parametric variables) or Mann-Whitney test (for non-parametric variables) were used to explore differences between growth (z-scores used as continuous variables) and age of solids introduction (coded as a binary variable: introduction of solids before versus at/after 6 months) and between growth and mode of complementary feeding (also coded as a binary variable: BLW versus spoon-fed).

Habitual intake of food types in grams (g) among consumers and energy and nutrient intakes were presented as means, standard deviations and selected points in the cumulative distribution, namely the 25th , 50th (median) and 75th percentiles. Unusual values for each nutrient were assessed on an individual basis. Wherever the nutrient intake was an unusual but plausible value, it was retained. For example there was an entry for mycoprotein which had unusually large content of certain micronutrients compared to other food items. Once the portion size was checked to be appropriate for the age group, the food item was included in the nutrient analysis. The same was true for nutrients whose value was extremely below recommended intakes, such as vitamin D. The majority of infants had very low Vitamin D intakes, therefore infants with Vitamin D intakes within the recommended range appeared as unusually high values when in fact they weren't, and thus were also retained in the analyses. If there was an implausible value, the data was reexamined for errors in data entry or nutrient analysis calculations. On 2 occasions the DINO programme had incorrectly reported values of micronutrients that were inconsistent with realistic intakes. The errors were traced back to the individual food item (infant formula in both cases) and for each entry of this item, the error was manually corrected using the manufacturer's nutrition information. Nutrient intakes were then recalculated.

For any age group, it is expected that around 50% will have requirements above the EAR and 50% will have requirements below the EAR, according to gender and age. At 8 months, individual EARs for energy for each infant were calculated according to gender and weight, using the revised energy reference values for mixed-fed infants. At 18 months, fixed EARs are set according to gender (Scientific Advisory Committee on Nutrition 2011a). For each infant, energy intakes were expressed as %EAR and mean protein, vitamin and mineral intakes as a proportion of the RNI. Nutrient adequacy was assessed by comparing the mean absolute (not energy-adjusted) intakes with the Dietary Reference Values (DRVs). For protein, vitamins and minerals, the proportion of infants with intakes below the Reference (RNI) and Lower Reference Nutrient Intake (LRNI) were calculated, where available. Differences in intakes between the complementary feeding groups and the baby-led weaning groups were assessed using independent samples t-test or Mann Whitney tests as appropriate. The differences in proportions of infants with nutrient intakes below the RNI and LRNI cut-offs between groups were assessed using Fisher's exact test.

Haemoglobin levels at 8 and 18 months were normally distributed. They were presented as means and medians with standard deviations and 95% confidence intervals, across the whole sample and stratified by age of solids introduction and mode of complementary feeding. Mean total Hb levels were compared in infants introduced to solids before and after six months and those who followed baby-led weaning or were spoon-fed using independent samples t-tests. Proportions of infants with haemoglobin levels below the WHO, ALSPAC and SACN cut-off points for anaemia were also calculated across groups, and compared using Fisher's exact tests. The association between the age of solids introduction (as a continuous variable) and haemoglobin levels at 8 and 18 months were assessed using Spearman rank correlation tests.

Energy adjusted total and non-haem iron intakes were compared between groups using t-tests and haem iron intakes were compared between groups using the Mann Whitney test. Absolute (i.e. not energy adjusted) mean values of total iron intakes were compared using the RNI, EAR and LRNI cut-offs. Differences in the proportion of infants below those cut-offs between groups were compared using Fisher's exact tests. The contribution of selected food groups to total, haem and non-haem iron intakes was calculated. At 8 months, the contribution to iron from formula milk, breast milk and all other sources was graphically represented. Associations between haemoglobin and the absolute and energy adjusted intakes of selected inhibitors and enhancers of iron absorption (total iron, haem iron, calcium, non-starch polysaccharides and vitamin C) were assessed using Pearson's correlation. Intakes of vitamin C and haem iron were transformed to the natural logarithm in order to normalize their distributions. Adjustment for gender was not necessary as haemoglobin did not differ significantly between boys and girls at either time point ($p=0.536$ and $p=0.251$ respectively).

Differences between various eating behaviours by the age of solids introduction and by mode of complementary feeding were established using Fisher's exact test because due to the small sample size, chi square assumptions on expected cell count were violated. Continuous variables such as age of introduction of purees, lumpy solids and finger foods were compared using Mann Whitney tests as they were not found to be normally distributed.

The level of significance was set at $p<0.05$.

3.2.10 Ethical Considerations

As with all research studies involving humans and especially due to the involvement of a vulnerable group such as infants in this particular project, there were a number of ethical considerations which were examined in order to obtain a favourable opinion from the local Research Ethics Committee.

The main ethical issue was the possible distress caused to the infant by taking a blood sample. However, it was argued that heel prick capillary blood samples are routinely taken from much younger infants without the use of anaesthetic and with no apparent prolonged adverse effects.

Possible distress could be caused to mothers if they were present when the blood was being taken, but it would be minimal as mothers are usually present when the infant has immunizations and are therefore familiar with such minor clinical procedures.

Distress could be caused to the mothers at the initial telephone contact if the infant had fallen ill, had passed away or if, for some reason, the mother had stopped breastfeeding. A self-addressed freepost envelope with a letter stating that they wish to withdraw from the study (without having to give a reason) was sent to the mothers as soon as they expressed interest during the recruitment period. They were advised to send it at any time point they felt they did not wish to continue with the study. It was made clear that they were not being judged for their decisions and that participation was entirely voluntary. As an additional measure to minimise possible distress, the health visitors' base was contacted to ask whether they knew of any deaths or serious illness in the families of the infants involved with the study.

If there was evidence from the results of the haemoglobin analysis that an infant was anaemic, it was agreed that the researcher would write to the mother, enclosing a letter for them to take to their general practitioner (GP).

Mothers might have felt pressurised by filling in the dietary records, or felt judged on the type of diets they feed to their infants. This was minimised by emphasizing that they were not being judged on this and that there were no "right or wrong" answers.

Some participants might not want to answer some of the questions on the demographic questionnaire. It was emphasized that questions may be omitted if they wished to do so.

Parents might be found to be feeding their children inappropriately. However a 3 day food record would not be sufficiently long in order to judge if a child was being fed appropriately (as the diet records might reflect atypical days), so no action could have been taken on the basis of this.

The growth measurements might have raised concern that the child was over- or underweight. However, it was considered that a one-off measurement of weight would not be sufficient to make judgements about growth. However if participants were worried about their babies' feeding or weight they were encouraged to see their GP or health visitor.

In the event of concerns for the welfare of the infant or others during home visits, the researcher would follow procedures in place applicable for members of public with regards to reporting these concerns. If there were concerns about the infant's health the mother would be encouraged to contact her GP or health visitor. If issues of abuse or neglect were suspected, the local Children's social care or the NSPCC would be contacted after consulting with the supervisory team or the police (if there was an immediate danger for harm). Participants were also given a reference to the Sussex child protection and safeguarding procedures document.

A possible risk for the researcher was visiting the participants in their homes. To minimise this risk, the researcher carried a mobile phone and always informed colleagues at the

University of Brighton where she was going and the anticipated return time. A second risk to the researcher was the possibility of contracting a blood transmitted infection. As the method of blood sampling was a heel prick the risk was minimal. However phlebotomy training ensured that all precautions were taken including good clinical practice procedures.

The study was approved by Kent Research Ethics Committee and Sussex NHS Research Consortium. The relevant documentation is included in Appendix 1.

3.3 Results

3.3.1 Participants

Twenty one participants were recruited of which one had to withdraw as her milk flow reduced substantially due to illness and was therefore no longer eligible to take part. All of the remaining participants (n=20) completed the first visit and second visit. Nineteen participants (95% retention rate) completed the 18 month visit (one participant withdrew due to lack of time). The very low number of participants recruited, the implications on the study results and their interpretation will be discussed in detail in the discussion section of this chapter as well as in Chapter 6.

3.3.2 Descriptive data

The demographic characteristics of the study group and the stratified groups are shown in Table 3-1. The average maternal age was 34.6 (SD: 4.2) years and 80% of mothers possessed a higher education qualification (degree or postgraduate qualification). Three quarters of them lived in owned or mortgaged properties and all lived with their partners. All infants were born to term and only 3 (15%) had birth weight below 3000g. However, all infants' birth weight was above 2500g (the cut-off point for low birth weight). There were no statistically significant differences in the socio-demographic characteristics between infants introduced to solids before or after 6 months and between infants following baby-led weaning or those being spoon-fed.

Table 3-1 Characteristics of the study group and stratified by age of solids introduction and by mode of complementary feeding

		Age of Solids Introduction			Mode of Complementary Feeding		
Variables	**All (n=20)**	<6 months (n=7)	≥6 months (n=13)	**P**	BLW (n=7)	Spoon-fed (n=13)	**P**
Infant characteristics							
Infant birth weight (g)	3501.5 (517.7)	3507.9 (240.5)	3498.1 (471)	0.969	3532.1 (574.8)	3485 (508.3)	0.852
Infant gender							
Male	11 (55)	4 (57.1)	7 (53.8)		4 (57.1)	7 (53.8)	
Female	9 (45)	3 (42.9)	6 (46.2)	1	3 (42.9)	6 (46.2)	1
Maternal characteristics							
Maternal age (y)	34.6 (4.2)	32.9 (2.8)	35.5 (4.5)	0.190	34.7 (3.5)	34.5 (4.6)	0.901
Maternal ethnic group							
White	19 (95)	6 (85.7)	13 (100)		7 (100)	12 (92.3)	
Other	1 (5)	1 (14.3)	0	0.350	0	1 (7.7)	1
Maternal education							
Postgraduate qualification	11 (55)	4 (57.1)	7 (53.8)	1	4 (57.1)	7 (53.8)	1
Degree	5 (25)	2 (28.6)	3 (23.1)	1	2 (28.6)	3 (23.1)	1
A levels	2 (10)	0	2 (15.4)	0.521	0	2 (15.4)	0.521
No qualifications	2 (10)	0	2 (15.4)	0.521	0	2 (15.4)	0.521
Housing tenure							
Being bought/mortgaged	13 (65)	6 (85.7)	7 (53.8)		3 (42.9)	10 (76.9)	
Owned- no mortgage to pay	2 (10)	0	2 (15.4)		1 (14.3)	1 (7.7)	

Variables	All (n=20)	Age of Solids Introduction			Mode of Complementary Feeding		
		<6 months (n=7)	≥6 months (n=13)	P	BLW (n=7)	Spoon-fed (n=13)	P
Rented	5 (25)	1 (14.3)	4 (30.8)	0.483	3 (42.9)	2 (15.4)	0.181
Maternal Smoking							
Current Smoker	1 (5)	1 (14.3)	0		0	1 (7.7)	
Former smoker	7 (35)	1 (14.3)	6 (46.2)		3 (42.9)	4 (30.8)	
Never smoked	12 (60)	5 (71.4)	7 (53.8)	0.146	4 (57.1)	8 (61.5)	1
Iron supplementation in pregnancy							
Yes	3 (15)	2 (28.6)	1 (7.7)		1 (14.3)	2 (15.4)	
No	16 (80)	5 (71.4)	11 (84.6)		6 (85.7)	10 (76.9)	
Yes, but didn't take it	1 (5)	0	1 (7.7)	0.690	0	1 (7.7)	1

Infant weight and maternal age: mean (SD); All other variables: n (%)

Maternal education: highest qualification

At 8 months all mothers (n=20) were still breastfeeding. The age of first solids introduction was not normally distributed. The age distribution by months is shown in Figure 3-2. The median age of solids introduction was 6 months (range 4-7 months, IQR: 5.5, 6). Four infants (20%) were introduced to solids before 5.5 months whereas one (5%) was introduced to solids at 7 months. Median age of solids introduction in infants following baby-led weaning was 6 months (IQR: 6, 6), whereas in the spoon-fed group the median age of solids introduction was 5.5 months (IQR: 5, 6). The age of solids introduction was significantly different between the two groups (p=0.030). There were also statistically significant differences between infants who were introduced to solids before six months (median: 5 months, IQR: 4.5, 5.5) and those introduced to solids after 6 months (median: 6 months, IQR: 6, 6)

Figure 3-2 Age of first solids introduction distribution of the study sample (n=20)

3.3.3 Growth

3.3.3.1 Growth patterns at birth and at 6 months

Weight and weight-for-age z-score at birth and at 6 months were normally distributed. Mean (SD) birth weight was 3.5kg (SD: 0.5) and the average weight-for-age z-score (WAZ) at birth was 0.39 (SD: 0.99). Mean weight at 6 months (mean age of measurement

6.15 months) was 7.68kg (SD: 1.07) and the average weight-for-age z-score was -0.08 (SD: 1.03). Table 3-2 provides a summary of the sample growth characteristics at birth and 6 months (weight for these two time points are recorded from the infants' Red Book, length was not available). There were no statistically significant differences in weight or weight-for-age z-score at birth or at six months of age between infants who were introduced to solids before or at/after 6 months and between those following baby-led weaning or those who were spoon-fed.

Table 3-2 Growth measurements at birth and at 6 months, across the whole sample and stratified by age of solids introduction and mode of complementary feeding

		Age of Solids Introduction			**Mode of Complementary Feeding**		
Growth Variables	**All (n=20)**	<6 months (n=7)	≥6 months (n=13)	**P**	BLW (n=7)	Spoon-fed (n=13)	**P**
Birth Weight (kg)	3.50 (0.5)	3.51 (0.64)	3.50 (0.47)	0.970	3.53 (0.57)	3.49 (0.51)	0.853
WAZ at birth	0.39 (0.99)	0.37 (1.15)	0.40 (0.94)	0.939	0.43 (1.14)	0.37 (0.94)	0.901
Weight at 6 months	7.68 (1.07)	7.47 (1.10)	7.80 (1.10)	0.519	7.99 (0.87)	7.52 (1.16)	0.353
WAZ at 6 months	-0.08 (1.03)	-0.30 (1.14)	0.05 (0.99)	0.490	0.28 (0.90)	-0.26 (1.10)	0.279

Abbreviations: WAZ: weight-for-age z-score

All variables: mean (SD)

3.3.3.2 Growth patterns at 8 months

The age of solids introduction (as a continuous variable) was positively correlated with weight-for-length z-score (Spearman's rho coefficient: 0.571, p=0.011) and BMI-for-age z-score (Spearman's rho coefficient: 0.611, p=0.05) at 8 months.

Growth patterns of infants at 8 months (mean age of measurement 8.17 months) are shown on Table 3-3. Mean weight was 8.49kg (SD: 1.09) and the average weight-for-age z-score was 0.08 (SD: 0.97). Mean length was 70.32cm (SD: 3.15) and average length-for-age z-score was 0.12 (SD: 1.22). All the variables above were normally distributed. In contrast, weight-for-length and BMI-for-age z-scores were not normally distributed. The median weight-for-length z-score was -0.26 (IQR: -0.99,-1.08) and the median BMI-for-age z-score was -0.26 (IQR: -0.96, 0.90). Infants who were introduced to solids before 6 months were lighter but on average almost 1cm longer than infants introduced to solids after 6 months, however there were no statistically significant differences observed in weight, weight-for-age z-score, length, length-for-age z-score or weight-for-length z-score between the two groups. However, infants in the early complementary feeding group had a statistically significant lower BMI-for-age z-score compared to infants in the later complementary feeding group (p=0.045). There were no statistically significant differences observed in weight, length, weight-for-age and length-for-age z-scores between infants who followed baby-led weaning and those who were spoon-fed. However, infants in the BLW group had a significantly higher weight-for-length z-score (p=0.028) and BMI-for-age z-score (p=0.022) than infants in the spoon-fed group.

Table 3-3 Growth measurements at 8 months, across the whole sample and stratified by age of solids introduction and mode of complementary feeding

		Age of Solids Introduction			**Mode of Complementary Feeding**		
Growth Variables	**All (n=20)**	<6 months (n=7)	≥6 months (n=13)	**P**	BLW (n=7)	Spoon-fed (n=13)	**P**
Weight (kg)	8.49 (1.09)	8.18 (2.15)	8.65 (1.07)	0.373	8.97 (0.85)	8.23 (1.15)	0.158
Length (cm)	70.32 (3.15)	70.86 (3.61)	70 (2.98)	0.583	69.64 (2.72)	70.71 (3.43)	0.493
WAZ	0.08 (0.97)	-0.16 (1.08)	0.22 (0.92)	0.419	0.51 (0.78)	-0.14 (1)	0.156
LAZ	0.12 (1.22)	0.56 (1.37)	-0.13 (1.12)	0.251	-0.27 (1.09)	0.36 (0.37)	0.298
WLZ	-0.26 (-0.99, -1.08)	-0.61 (-0.94,-0.38)	0.54 (-0.61, 1.49)	0.068	1.08 (0.41,1.74)	-0.54 (-0.93,0.04)	**0.028**
BMZ	-0.26 (-0.96,0.90)	-0.90 (-1.16,-0.50)	0.48 (-0.73, 1.47)	**0.045**	0.90 (0.37,1.76)	-0.78 (-1,-0.04)	**0.022**

Abbreviations: WAZ: weight-for-age z-score; LAZ: length-for-age z-score; WLZ: weight-for-length z-score; BMZ: BMI-for-age z-score;

Weight, Length, WAZ, LAZ: mean (SD:); WLZ, BMZ: median and (interquartile range)

For Length, LAZ, WLZ, BMZ: n=19 due to missing data (< 6months: n=6, ≥6 months n=13, BLW: n=7, spoon-fed: n=12)

3.3.3.3 Growth patterns at 18 months

At 18 months of age, no significant correlations were observed between the age of solids introduction and any of the growth variables (mean age of measurement 17.98 months). Mean weight across the sample was 10.91kg (SD: 1.21) and average weight-for-age z-score was 0.17 (SD: 0.84). Mean length was 81.79 cm (SD: 3.94) and the average z-score length z-score for age was 0.09 (SD: 1.35). Mean BMI-for-age z-score was 0.19 (SD: 0.80). All these variables were normally distributed. Average weight-for-length z-score was 0.19 (IQR -0.33, 0.51) and was not normally distributed. Infants who were introduced to solids at/after 6 months continued to be heavier than those introduced to solids before 6 months although both groups were within the healthy growth range and there were no significant differences between them. Infants in the BLW group had significantly higher weight-for-length z-score (p=0.028) and BMI-for-age z-score (p=0.020) than those in the spoon-fed group, as they did at 8 months. No other differences were observed in the BLW and spoon-fed group at 18 months. The 18 month growth data are summarised in Table 3-4.

Table 3-4 Growth measurements at 18 months, across the whole sample and stratified by age of solids introduction and mode of complementary feeding

		Age of Solids Introduction			**Mode of Complementary Feeding**		
Growth Variables	**All (n=19)**	<6 months (n=6)	≥6 months (n=13)	**P**	BLW (n=7)	Spoon-fed (n=12)	**P**
Weight (kg)	10.91 (1.21)	10.78 (1.25)	10.97 (1.25)	0.760	11.48 (1.48)	10.57 (0.94)	0.117
Length (cm)	81.79 (3.94)	82.33 (3.72)	81.54 (4.16)	0.695	82 (1.97)	81.67 (3.45)	0.865
WAZ	0.17 (0.84)	0.01 (0.87)	0.24 (0.86)	0.601	0.57 (1)	-0.07 (0.68)	0.114
LAZ	0.09 (1.35)	0.20 (1.18)	0.04 (1.47)	0.819	0.12 (1.73)	0.07 (1.17)	0.931
WLZ	0.19 (-0.33,0.51)	-0.04 (-0.61, 0.38)	0.19 (-0.30, 0.65)	0.416	0.28 (0.09, 1.26)	-0.30 (-0.50,0.29)	**0.028**
BMZ	0.19 (0.80)	-0.12 (0.51)	0.33 (0.89)	0.267	0.73 (0.94)	-0.13 (0.53)	**0.020**

Abbreviations: WAZ: weight-for-age z-score; LAZ: length-for-age z-score; WLZ: weight-for-length z-score; BMZ: BMI-for-age z-score;

Weight, Length, WAZ, LAZ, BMZ: mean (SD:); WLZ: median and (interquartile range)

3.3.4 Diet

3.3.4.1 Food Groups at 8 months

Daily consumption of various food groups at 8 months is presented in Table 3-5. Overall, among consumers, breast milk was consumed in large quantities (mean consumption of 495g per day), followed by fruit (mean consumption of 95.5g per day), milk and milk products (mean consumption of 82g per day) and vegetables and potatoes (mean consumption of 70.4g). All infants were consuming breast milk and cereal/cereal dishes by 8 months of age, followed by 19 infants (95%) consuming vegetables and potatoes. In terms of infant specific foods, 8 infants (40%) consumed formula whereas 7 infants (35%) consumed commercial infant foods.

Table 3-5 Daily consumption of selected foods from dietary records, at 8 months

		Daily consumption (g)			
Food group[1]	**Consumers (n)**	**Minimum**	**Maximum**	**Mean**	**SD**
Cereals and cereal dishes	20	2	153.2	39.4	37.8
Milk and dairy products	18	9	212.9	84.2	57
Meat and meat products	12	1	49.8	21.7	16.3
Fish and fish dishes	6	7.5	35	15.6	9.9
Eggs and egg dishes	10	1.4	120	37	34.7
Vegetables and potatoes	19	7.1	192.4	70.4	48.3
Lentils and pulses	5	12.6	62.9	37	22.2
Fruit	20	5.8	222.1	95.5	62.8
Infant formula	8	1.66	270.7	152.8	89.2
Breast milk[2]	20	203.3	966.7	495.2	208.6
Commercial infant foods	7	12	285	11.8	91.1

[1] Some food groups are not included due to small numbers of consumers; e.g. nuts and savoury snacks

[2] Typical volumes of breast milk for full feeds were estimated from MRC data (Paul et al. 1988) to be approximately 100g for those aged 8 months. It was assumed that a feed of 10 minutes or longer was equivalent to a full feed. For feeds of less than 10 minutes duration weights were calculated proportionately at 10g/min for 8 months.

3.3.4.2 Nutrient intakes at 8 months

Nutrient intakes and distributions across the 25-75th centiles at 8 months across the whole sample are presented in Table 3-6. Mean intakes of energy and most other nutrients were above the recommended nutrient intakes including energy in boys, protein, thiamine, B6, B12, folate, vitamin C, vitamin A, magnesium, sodium potassium, copper selenium and iodine. Mean energy intakes in girls, niacin and phosphorus were very only marginally below the recommended intakes. However, mean calcium and zinc intakes were lower than recommended intakes (81% and 76% of the RNI respectively), whereas iron and vitamin D intakes were much lower than recommended intakes (42% and 23% of the RNI respectively). It is worth noting that Vitamin D intakes do not include intakes from breast milk (as its Vitamin D content is not known) and therefore are an underestimate of actual intakes.

Table 3-6 Average daily intake and distributions across 25th -75th centiles of energy, macronutrients, vitamins and minerals, across the whole sample at 8 months

	All infants (n=20)					
Nutrient /day	**Mean**	**SD**	**25th Centile**	**Median**	**75th centile**	**Mean as %RNI**
Energy KJ (males) [1]	2970	300	2633	2996	3214	105
Energy kcal (males) [1]	711	72	630	717	769	105
Energy KJ (females)[2]	2580	329	2368	2585	2844	99
Energy kcal (females)[2]	613	78	563	615	676	98
Protein (g)	20.7	3.1	17.9	20.3	23.0	151
Total fat (g)	36	4	33	37	39	-
Total Carbohydrate (g)	92	10	85	93	99	-
Total NSP (g)	5	2	4	5	7	-
Saturated Fats (g)	14	2	13	14	16	-
Mono-unsaturated fats (g)	13	3	12	13	15	-

	All infants (n=20)					
Nutrient /day	**Mean**	**SD**	**25th Centile**	**Median**	**75th centile**	**Mean as %RNI**
Poly-unsaturated fats (g)	5	1	4	5	5	-
Thiamine (mg)	0.5	0.1	0.4	0.5	0.6	240
Riboflavin (mg)	0.6	.2	0.4	0.5	0.7	144
Niacin (mg)	4.5	1.5	3.4	4.0	5.8	99
Pyridoxine B6 (mg)	0.6	0.2	0.4	0.6	0.7	274
B12 (µg)	0.9	0.6	0.4	0.7	1.2	217
Folate (µg)	98	30	74	90	116	197
Vitamin C (mg)	57	21	43	57	73	226
Vitamin A (µg) (retinol equivalent)	693	185	558	631	834	198
Vitamin D (µg)	1.6	1.7	0.5	0.9	1.8	23
Calcium (mg)	424	99	356	398	458	81
Phosphorus (mg)	367	77	315	365	408	92
Magnesium (mg)	88	15	78	87	99	117

	All infants (n=20)					
Nutrient /day	**Mean**	**SD**	**25th Centile**	**Median**	**75th centile**	**Mean as %RNI**
Sodium (mg)	417	284	273	351	505	130
Potassium (mg)	1089	191	942	1098	1265	156
Chloride (mg)	787	418	552	711	924	157
Iron (mg)	3.2	1.3	1.9	3.0	4.1	42
Zinc (mg)	3.8	0.7	3.2	3.6	4.3	76
Copper (mg)	0.5	0.1	0.5	0.5	0.6	177
Selenium (µg)	14	3	11	14	15	137
Iodine (µg)	72	16	59	72	80	121

[1] For Energy intakes in boys n=11

[2] For Energy intakes in girls n=9

3.3.4.3 Nutrient intakes at 8 months by age of solids introduction

Table 3-7 shows mean nutrient intakes and their distribution across 25th-75th centiles at 8 months stratified by timing of introduction of solids (before or after 6 months). Infants who were introduced to solids after 6 months (n=13) had significantly lower intakes of riboflavin (p=0.032), Vitamin B6 (p=0.031), Vitamin B12 (p=0.019) and selenium (p=0.014) than infants who were introduced to solids before 6 months (n=7). Looking at mean intakes as %RNI, for the vast majority of nutrients in both groups, intakes exceeded the RNI. Girls who were introduced to solids before 6 months had energy intakes below the estimate average requirements (93% EAR) compared to girls who were introduced to solids after 6 months (106% EAR), although the difference was not statistically significant (p=0.360). Vitamin D, calcium, zinc and iron intakes were below requirements in both groups even though the differences between the groups were not statistically significant. Phosphorus intakes in infants introduced to solids after 6 months were below the RNI (86% of RNI) whereas infants who were introduced to solids before 6 months had phosphorus intakes just above the recommended intake (102% of the RNI). However the differences in intakes between the two groups were not statistically significant.

Table 3-7 Average daily intake and distributions across 25th -75th centiles of energy, macronutrients, vitamins and minerals, stratified by age of solids introduction at 8 months

	<6 months (n=7)						≥6 months (n=13)						
Nutrient /day	**Mean**	**SD**	**25th Centile**	**Median**	**75th centile**	**Mean as %RNI**	**Mean**	**SD**	**25th centile**	**Median**	**75th centile**	**Mean as %RNI**	**P**
Energy KJ (boys) [1]	2894	282	2615	2911	3156	102	3013	324	2633	3016	3359	106	0.555
Energy kcal (boys) [1]	693	67	626	697	756	102	721	77	630	722	804	106	0.555
Energy KJ (girls)[2]	2427	373	2001	2585	-	93	2656	311	2447	2585	3003	102	0.360
Energy kcal (girls)[2]	577	89	476	615	-	93	632	74	582	615	714	101	0.360
Protein (g)	22.4	3.7	20.4	22.5	26.1	164	19.8	2.5	17.5	19.3	22.3	145	0.081
Total fat (g)	34	3	32	33	38	-	37	5	34	38	40	-	0.128
Saturated Fats (g)	14	2	13	14	16	-	15	2	13	14	17	-	0.659
MUFA (g)	12	3	10	12	13	-	13	2	12	13	15	-	0.230
PUFA (g)	4	1	4	4	5	-	5	1	4	5	6	-	0.062
Total CHO (g)	95	8	87	98	100	-	91	10	85	89	99	-	0.331
Total NSP (g)	5	1	4	4	7	-	5	2	4	5	7	-	0.676

	<6 months (n=7)						≥6 months (n=13)						
Nutrient /day	**Mean**	**SD**	**25^{th} Centile**	**Median**	**75^{th} centile**	**Mean as %RNI**	**Mean**	**SD**	**25^{th} centile**	**Median**	**75^{th} centile**	**Mean as %RNI**	**P**
Thiamine (mg)	0.5	0.1	0.4	0.6	0.6	282	0.5	0.2	0.3	0.5	0.6	217	0.423
Riboflavin (mg)	0.7	0.2	0.5	0.6	0.9	173	0.5	0.2	0.4	0.4	0.7	129	**0.032**
Niacin (mg)	5.4	1.3	4.0	5.5	6.6	126	4.0	1.5	2.9	3.6	5.2	84	0.058
Pyridoxine B6 (mg)	0.7	0.2	0.6	0.7	0.7	317	0.5	0.2	0.4	0.5	0.6	251	**0.031**
B12 (µg)	1.2	0.6	0.8	1.0	2.0	305	0.7	0.5	0.4	0.6	0.8	169	**0.019**[3]
Folate (µg)	97	21	74	93	117	194	99	35	75	85	114	198	0.699[3]
Vitamin C (mg)	60	19	50	62	78	240	55	22	40	50	69	219	0.599
Vitamin A (µg)	736	186	599	730	867	210	670	188	557	618	751	191	0.462
Vitamin D (µg)	2.3	2.2	0.9	1.1	5.2	32	1.2	1.4	0.4	0.6	1.7	18	0115[3]
Calcium (mg)	455	142	342	410	582	87	408	68	357	385	449	78	0.757[3]
Phosphorus (mg)	408	93	318	393	466	102	344	60	277	357	390	86	0.078
Magnesium (mg)	89	9	80	90	100	119	87	18	70	86	99	115	0.721
Sodium (mg)	443	212	276	369	577	139	403	324	228	336	442	126	0.536[3]

Nutrient /day	<6 months (n=7)						≥6 months (n=13)						P
	Mean	SD	25th Centile	Median	75th centile	Mean as %RNI	Mean	SD	25th centile	Median	75th centile	Mean as %RNI	
Potassium (mg)	1168	186	1121	1157	1314	167	1047	187	920	990	1156	150	0.186
Chloride (mg)	817	311	570	698	959	163	771	478	489	724	856	154	0.643[3]
Iron (mg)	3.7	1.4	2.6	3.1	5.2	47	3.0	1.2	1.8	3.0	4.0	39	0.317
Zinc (mg)	4.0	0.9	3.1	4.0	4.6	80	3.7	0.6	3.3	3.5	4.0	74	0.341
Copper (mg)	0.5	0.1	0.4	0.5	0.6	168	0.5	0.1	0.5	0.5	0.6	182	0.282
Selenium (μg)	16	4	14	16	18	159	13	2	11	12	14	125	**0.014**
Iodine (μg)	81	19	68	79	104	135	68	12	58	67	77	113	0.064

[1] For energy intakes in boys n=4 before 6 months, n=7 after 6 months

[2] For energy intakes in girls n=3 before 6 months, n=6 after 6 months

[3] Mann Whitney test

Abbreviations: CHO: carbohydrate, NSP: Non-starch polysaccharides, MUFA: Mono-unsaturated fatty acids, PUFA: Poly-unsaturated fatty acids

3.3.4.4 Nutrient intakes at 8 months by mode of complementary feeding

Looking at mean nutrient intakes stratified by method of complementary feeding (Table 3-8), infants who followed baby-led weaning (n=7) had significantly higher intakes of polyunsaturated fatty acids (p=0.002) than those who were spoon-fed (n=13). However, the baby-led weaning group had significantly lower riboflavin, Vitamin D and Iodine intakes (p=0.036, p=0.037 and p=0.017 respectively) than the spoon-fed group at 8 months. The majority of mean nutrients were above the recommended intakes in both groups. Girls who were spoon-fed had energy intakes lower than the EAR (93%) as opposed to girls who followed baby-led weaning (109%), however the difference did not reach statistical significance (p=0.075). Mean niacin intakes were below the RNI in infants who were baby-led weaned (93%) whereas their spoon-fed counterparts had intakes just over the RNI (102%). Again, the difference was not significant. Of the remaining mean nutrient intakes in both groups, namely phosphorus, calcium, iron and zinc, the spoon-fed group had higher intakes than the baby-led weaned group but none of the differences were statistically significant.

Table 3-8 Average daily intake and distributions across 25th -75th centiles of energy, macronutrients, vitamins and minerals, stratified by method of complementary feeding at 8 months

	BLW (n=7)						Spoon-fed (n=13)						
Nutrient /day	**Mean**	**SD**	**25th Centile**	**Median**	**75th centile**	**Mean as %RNI**	**Mean**	**SD**	**25th centile**	**Median**	**75th centile**	**Mean as %RNI**	**P**
Energy KJ (boys) [1]	3026	300	2729	3056	3293	107	2938	320	2574	2983	3214	104	0.664
Energy kcal (boys) [1]	724	72	653	732	788	107	703	77	616	714	769	103	0.664
Energy KJ (girls) [2]	2851	283	2526	2991	-	109	2444	274	2158	2578	2622	94	0.075
Energy kcal (girls) [2]	678	67	601	711	-	109	581	65	513	613	624	93	0.075
Protein (g)	20.0	2.7	17.5	19.3	23.1	146	21.2	3.4	18.1	21.6	23.1	154	0.432
Total fat (g)	38	4	34	38	41	-	35	4	32	35	39	-	0.109
Saturated Fats (g)	14	2	13	14	16	-	14	2	12	14	17	-	0.907
MUFA (g)	14	2	13	13	15	-	12	3	10	12	14	-	0.176
PUFA (g)	6	1	5	6	7	-	4	1	4	5	5	-	**0.002**

	BLW (n=7)						Spoon-fed (n=13)						
Nutrient /day	**Mean**	**SD**	**25th Centile**	**Median**	**75th centile**	**Mean as %RNI**	**Mean**	**SD**	**25th centile**	**Median**	**75th centile**	**Mean as %RNI**	**P**
Total CHO (g)	88	10	85	89	95	-	94	9	86	98	99	-	0.152
Total NSP (g)	6	2	4	7	7	-	5	2	4	4	7	-	0.293
Thiamine (mg)	0.5	0.1	0.3	0.5	0.5	224	0.5	0.1	0.4	0.6	0.6	248	0.391
Riboflavin (mg)	0.5	0.1	0.4	0.4	0.6	116	0.6	0.2	0.5	0.6	0.8	159	0.036
Niacin (mg)	4.2	1.5	3.4	3.7	4.9	93	4.6	1.6	3.1	4.4	6.0	102	0.580
Pyridoxine B6 (mg)	0.5	0.1	0.4	0.5	0.6	249	0.6	0.2	0.4	0.6	0.7	288	0.278
B12 (µg)	0.7	0.3	0.4	0.6	0.8	164	1.0	0.6	0.4	0.8	1.6	245	0.351[3]
Folate (µg)	100	35	73	93	116	200	97	29	75	87	116	195	1[3]
Vitamin C (mg)	56	17	42	50	75	224	57	23	36	59	72	227	0.940
Vitamin A (µg)	700	235	557	614	933	200	690	163	579	681	812	197	0.907
Vitamin D (µg)	0.7	0.6	0.4	0.5	1.2	11	2.0	2.0	0.7	1.1	3.8	29	0.037[3]
Calcium (mg)	378	36	355	367	421	72	449	114	350	413	530	86	0.241[3]
Phosphorus (mg)	327	65	264	330	380	82	388	78	337	374	418	97	0.084

Nutrient /day	BLW (n=7)						Spoon-fed (n=13)						P
	Mean	SD	25th Centile	Median	75th centile	Mean as %RNI	Mean	SD	25th centile	Median	75th centile	Mean as %RNI	
Magnesium (mg)	89	19	70	88	102	119	86	14	78	86	97	115	0.701
Sodium (mg)	555	378	365	389	562	173	343	198	218	286	439	107	0.056[3]
Potassium (mg)	1078	172	961	1057	1166	154	1095	207	920	1121	1307	156	0.857
Chloride (mg)	991	559	724	787	948	198	678	290	467	659	825	136	0.067[3]
Iron (mg)	3.0	1.0	1.8	3.1	3.8	38	3.4	1.5	2.0	3.0	5.0	43	0.545
Zinc (mg)	3.5	0.4	3.2	3.5	3.7	71	3.9	0.8	3.2	3.8	4.5	79	0.222
Copper (mg)	0.6	0.1	0.5	0.5	0.6	187	0.5	0.1	0.5	0.5	0.6	172	0.250
Selenium (µg)	13	2	10	14	15	127	14	3	12	14	17	142	0.304
Iodine (µg)	62	8	57	61	67	103	78	16	69	78	86	131	**0.017**

[1] For energy intakes in boys n=4 in BLW group, n=7 in spoon-fed group

[2] For energy intakes in girls n=3 in BLW group, n=6 in spoon-fed group

[3] Mann Whitney test

Abbreviations: CHO: carbohydrate, NSP: Non-starch polysaccharides, MUFA: Mono-unsaturated fatty acids, PUFA: Poly-unsaturated fatty acids, equiv: equivalents

3.3.4.5 Nutrient comparisons with DRVs at 8 months

At 8 months 23.7% of boys and 66.7% of girls had energy intakes below the estimated average requirements. In terms of vitamins, all infants had intakes below the RNI for vitamin D, 35% had intakes below the RNI for riboflavin and 65% infants had intakes below the RNI for niacin. In terms of mineral intakes, iron fared the worst with all infants below the RNI and 70% below the LRNI, followed by zinc with 85% of infants below the RNI; however in the case of zinc 40% of the sample had intakes below the LRNI. Calcium and phosphorus intakes were below the RNI for 75% and 55% of the sample respectively, with 20% infants below the LRNI for each nutrient. Sodium intakes were low for 45% of the sample and 15% of them had intakes below the LRNI. Iodine and magnesium intakes were below the RNI for 40% of the sample, with 5% infants below the LRNI for Iodine but not magnesium. Finally selenium and chloride intakes were below the RNI for 30% and 20% of the sample respectively. There were no statistically significant differences in the proportion of infants below the RNI and LRNI between infants introduced to solids before or after six months. The only significant difference when looking at mode of complementary feeding was a significantly higher proportion of BLW infants with low riboflavin intakes compared to spoon-fed infants (p=0.022) (Tables 3-9 and 3-10).

Table 3-9 Proportion of participants with average daily intake of energy, macronutrients, vitamins and minerals below the Reference Nutrient intake (RNI) across the sample and stratified by age of solids introduction and mode of complementary feeding at 8 months

		Age of Solids Introduction			**Mode of Complementary Feeding**		
Nutrient /day	**All (n=20)**	<6 months (n=7)	≥6 months (n=13)	**P**	BLW (n=7)	Spoon-fed (n=13)	**P**
Energy (kcal) (boys)	3 (27.3)	1 (25)	2 (28.6)	1	1 (25)	2 (28.6)	1
Energy (kcal) (girls)	6 (66.7)	3 (100)	3 (50)	1	1 (33.3)	5 (83.3)	
Protein (g)	4 (20)	1 (14.3)	3 (23.1)	1	2 (28.8)	2 (15.4)	0.587
Thiamine (mg)	1 (5)	0	1 (7.7)	1	1 (14.3)	0	0.350
Riboflavin (mg)	7 (35)	1 (14.3)	6 (46.2)	0.329	5 (71.4)	2 (15.4)	**0.022**
Niacin (mg)	13 (65)	3 (42.9)	10 (76.9)	0.174	6 (85.7)	7 (53.8)	0.329
B12 (μg)	4 (20)	1 (14.3)	3 (23.1)	1	1(14.3)	3 (23.1)	1
Folate (μg)	2 (10)	0	2 (15.4)	0.521	1(14.3)	1 (7.7)	1
Vitamin C (mg)	2 (10)	1 (14.3)	1 (7.7)	1	1(14.3)	1 (7.7)	1
Vitamin D (μg)	20(100)	7 (100)	13 (100)	-	7(100)	13(100)	
Calcium (mg)	15 (75)	5 (71.4)	10 (76.9)	0.594	6 (85.7)	9 (69.2)	0.406
Phosphorus (mg)	11 (55)	4 (57.1)	7 (53.8)	1	5 (71.4)	6 (46.2)	0.374

Nutrient /day	All (n=20)	Age of Solids Introduction			Mode of Complementary Feeding		
		<6 months (n=7)	≥6 months (n=13)	**P**	BLW (n=7)	Spoon-fed (n=13)	**P**
Magnesium (mg)	8 (40)	4 (57.1)	4 (30.8)	0.356	3(42.9)	5 (38.5)	0.608
Sodium (mg)	9 (45)	3 (42.9)	6 (46.2)	1	2 (28.6)	7 (53.8)	0.374
Potassium (mg)	5 (25)	2 (28.6)	3 (23.1)	1	2 (28.6)	3 (23.1)	1
Chloride (mg)	4 (20)	2 (28.6)	2 (15.4)	0.587	1 (14.3)	3 (23.1)	1
Iron (mg)	20 (100)	7 (100)	13 (100)	-	7 (100)	13 (100)	-
Zinc (mg)	17 (85)	7 (100)	10 (76.9)	0.521	6 (85.7)	11 (84.6)	1
Copper (mg)	2 (10)	1 (14.3)	1 (7.7)	1	1 (14.3)	1 (7.7)	1
Selenium (µg)	6 (30)	2 (28.6)	4 (30.8)	1	3 (42.9)	3 (23.1)	0.613
Iodine (µg)	8 (40)	2 (28.6)	6 (46.2)	0.642	5 (71.4)	3 (23.1)	0.062

All variables n (%), EAR for energy

[1] For energy intakes in boys n=4 in BLW group, n=7 in spoon-fed group, n=4 before 6 months, n=7 after 6 months
[2] For energy intakes in girls n=3 in BLW group, n=6 in spoon-fed group, n=3 before 6 months, n=6 after 6 months

Table 3-10 Proportion of participants with average daily intake of energy, macronutrients, vitamins and minerals below the Lower Reference Nutrient in take (LRNI) across the sample and stratified by age of solids introduction and mode of complementary feeding at 8 months

		Age of Solids Introduction			**Mode of Complementary Feeding**		
Nutrient /day	**All (n=20)**	<6 months (n=7)	≥6 months (n=13)	P	BLW (n=7)	Spoon-fed (n=13)	P
B12 (μg)	2 (10)	0	2 (15.4)	0.521	1 (14.3)	1 (7.7)	1
Calcium (mg)	2 (10)	1 (14.3)	1 (7.7)	1	1 (14.3)	1 (7.7)	1
Phosphorus (mg)	2 (10)	0	2 (15.4)	0.521	1 (14.3)	1 (7.7)	1
Sodium (mg)	3 (15)	1 (14.3)	2 (15.4)	1	1 (14.3)	2 (15.4)	1
Chloride (mg)	1 (5)	0	1 (7.7)	1	1 (14.3)	0	0.350
Iron (mg)	14 (70)	5 (71.4)	9 (69.2)	1	6 (85.7)	8 (61.5)	0.354
Zinc (mg)	8 (40)	3 (42.9)	5 (38.5)	11	4	4 (30.8)	0.356
Iodine (μg)	1 (5)	1 (14.3)	0	0.350	0	1 (7.7)	1

All variables n (%)

3.3.4.6 Food Groups at 18 months

Daily consumption of various food groups at 18 months is presented in Table 3-11. Overall, among consumers, milk and dairy products and fruit were consumed in large quantities (mean of 320g and 195g per day respectively) followed by cereal products (mean consumption 130g per day) and vegetables and potatoes (mean consumption 108.9g). All infants were consuming cereal and cereal dishes, milk and dairy products, fruit and beverages (excluding water) by 18 months of age, followed by 18 infants (95%) consuming vegetables and potatoes, 13 (68%) meat and meat products, 11 (58%) eggs and egg dishes, lentils and pulses. Nine infants (47%) were still breastfeeding, however in terms of infant specific foods only 1 infant (5%) consumed formula whereas 3 infants (16%) consumed commercial infant foods.

Table 3-11 Daily consumption of selected foods from dietary records, at 18 months

		Daily consumption (g)			
Food group[1]	**Consumers (n)**	**Minimum**	**Maximum**	**Mean**	**SD**
Cereals and cereal dishes	19	40.7	203.7	130.3	45.2
Milk and dairy products	19	47.5	767.3	320.5	219.6
Meat and meat products	13	16.3	182.0	59.3	44.6
Fish and fish dishes	11	2.7	106.0	37.4	29.6
Eggs and egg dishes	11	14.2	120.0	53.5	27.8
Vegetables and potatoes	18	9.0	218.5	114.9	54.0
Lentils and pulses	11	11.8	200.0	67.5	51.9
Fruit	19	44.7	466.7	195.3	126.0
Nuts	9	6.5	60.0	22.6	16.9
Savoury snacks	15	2.0	63.5	15.1	15.1

Food group[1]	Consumers (n)	Daily consumption (g)			
		Minimum	Maximum	Mean	SD
Beverages	19	14.0	220.0	86.4	72.2
Infant formula	1	156.7	156.7	156.7	
Breast milk[2]	9	100.0	760.0	287.8	212.9
Commercial infant foods	3	106.8	141.7	124.5	17.4

[1] Some food groups are not included due to small numbers of consumers; e.g soups

[2] Typical volumes of breast milk for full feeds were estimated from MRC data (Paul et al. 1988) to be approximately 100g for those aged 8 months. It was assumed that a feed of 10 minutes or longer was equivalent to a full feed. For feeds of less than 10 minutes duration weights were calculated proportionately at 10g/min for 8 months.

3.3.4.7 Nutrient intakes at 18 months

Nutrient intakes and distributions across the 25-75th centiles at 18 months across the whole sample are presented in Table 3-12. Mean intakes of energy and almost all other nutrients were above the recommended nutrient but intakes of vitamin D were still much lower than recommended intakes (31% of the RNI) and iron intakes were marginally lower (96%) than the RNI. It is worth noting again that Vitamin D intakes do not include intakes from breast milk and therefore are an underestimate of actual intakes, however to a lesser extent than that of the 8 month time point, as fewer infants were still breastfeeding at 18 months.

Table 3-12 Average daily intake and distributions across 25th -75th centiles of energy, macronutrients, vitamins and minerals, across the whole sample at 18 months

	All infants (n=19)					
Nutrient /day	**Mean**	**SD**	**25th Centile**	**Median**	**75th centile**	**Mean as %RNI**
Energy KJ (males) [1]	5209	1074	4005	5269	5681	140
Energy kcal (males) [1]	1239	255	950	1252	1350	141
Energy KJ (females)[2]	4835	983	4154	4411	5918	140
Energy kcal (females)[2]	1149	236	989	1047	1409	139
Protein (g)	42.2	8.8	36.9	41.2	45.0	291
Total fat (g)	51	8	45	49	56	-
Saturated Fats (g)	21	5	16	22	25	-
Mono-unsaturated fats (g)	16	4	14	15	19	-
Poly-unsaturated fats (g)	8	4	5	7	10	-
Total Carbohydrate (g)	153	21	136	152	169	-
Total NSP (g)	12	3	9	11	14	-
Thiamine (mg)	0.9	0.2	0.8	0.9	1.1	204

	All infants (n=19)					
Nutrient /day	Mean	SD	25th Centile	Median	75th centile	Mean as %RNI
Riboflavin (mg)	1.3	0.4	0.9	1.2	1.7	219
Niacin (mg)	8.8	2.2	6.8	9.1	10.1	114
Pyridoxine B6 (mg)	1.2	0.2	1.1	1.2	1.3	195
B12 (μg)	3.0	1.4	1.8	2.4	4.3	599
Folate (μg)	180	38	155	179	213	257
Vitamin C (mg)	78	29	59	77	93	260
Vitamin A (μg) (retinol equivalent)	658	261	431	656	815	164
Vitamin D (μg)	2.1	1.7	1.0	1.8	2.6	31
Calcium (mg)	758	246	517	779	968	217
Phosphorus (mg)	853	200	666	874	1005	316
Magnesium (mg)	193	49	157	178	225	227
Sodium (mg)	1210	367	995	1193	1374	242
Potassium (mg)	2052	411	1825	2118	2319	256
Chloride (mg)	1948	518	1676	1922	2166	243

	All infants (n=19)					
Nutrient /day	**Mean**	**SD**	**25th Centile**	**Median**	**75th centile**	**Mean as %RNI**
Iron (mg)	6.6	1.6	5.0	6.3	8.0	96
Zinc (mg)	5.9	1.2	5.3	5.5	6.3	117
Copper (mg)	0.8	0.3	0.6	0.8	1.0	196
Selenium (µg)	26	10	21	23	30	174
Iodine (µg)	152	64	95	130	204	218

[1] For Energy intakes in boys n=11

[2] For Energy intakes in girls n=8

3.3.4.8 Nutrient intakes at 18 months by age of solids introduction

Table 3-13 shows mean nutrient intakes and their distribution across 25th-75th centiles at 18 months stratified by timing of introduction of solids (before or after 6 months). Infants who were introduced to solids after 6 months (n=13) had significantly lower intakes of saturated fatty acids (p=0.005) than infants who were introduced to solids before 6 months (n=6). Looking at mean intakes as %RNI (Table 3-13) for the vast majority of nutrients in both groups, intakes exceeded the RNI. Vitamin D and -to a much lesser extent- iron intakes were below requirements in both groups even though the differences between the groups were not statistically significant.

Table 3-13 Average daily intake and distributions across 25th -75th centiles of energy, macronutrients, vitamins and minerals, stratified by age of solids introduction at 18 months

	<6 months (n=6)						≥6 months (n=13)						
Nutrient /day	**Mean**	**SD**	**25th Centile**	**Median**	**75th centile**	**Mean as %RNI**	**Mean**	**SD**	**25th centile**	**Median**	**75th centile**	**Mean as %RNI**	**P**
Energy KJ (boys) [1]	4952	699	4234	5230	5392	133	5356	1269	4005	5569	6629	144	0.576
Energy kcal (boys) [1]	1179	166	1008	1243	1285	134	1274	302	950	1319	1583	145	0.581
Energy KJ (girls)[2]	4523	572	4118	4523	-	131	4939	1112	4159	4411	6298	143	0.642
Energy kcal (girls)[2]	1079	137	982	1079	-	131	1173	267	985	1047	1499	142	0.661
Protein (g)	46.1	9.3	38.9	43.8	53.5	318	40.4	8.3	36.2	39.4	42.5	279	0.179[3]
Total fat (g)	55	5	50	55	60		49	8	44	46	56		0.129
Saturated Fats (g)	25	1	23	24	26		19	6	15	18	23		**0.005**
MUFA (g)	17	3	14	18	19		16	4	14	15	19		0.698
PUFA (g)	7	2	6	6	8		8	4	5	8	11		0.388

Nutrient /day	<6 months (n=6)						≥6 months (n=13)						P
	Mean	**SD**	**25th Centile**	**Median**	**75th centile**	**Mean as %RNI**	**Mean**	**SD**	**25th centile**	**Median**	**75th centile**	**Mean as %RNI**	**P**
Total CHO (g)	139	11	130	138	146		159	22	145	161	179		0.054
Total NSP (g)	10	2	9	10	12		12	3	10	11	14		0.282[3]
Thiamine (mg)	1.0	0.2	0.8	0.9	1.2	220	0.9	0.2	0.7	0.9	1.1	196	0.464
Riboflavin (mg)	1.4	0.4	1.0	1.6	1.7	241	1.3	0.5	0.9	1.1	1.7	209	0.384
Niacin (mg)	8.9	2.0	7.2	8.6	10.2	118	8.7	2.3	6.4	9.1	10.1	113	0.907
Pyridoxine B6 (mg)	1.2	0.1	1.1	1.2	1.3	174	1.2	0.3	1.1	1.2	1.4	205	0.845
B12 (μg)	3.6	1.3	2.2	4.1	4.6	723	2.7	1.4	1.7	2.4	4.2	542	0.200
Folate (μg)	170	39	146	176	199	243	185	39	149	190	214	264	0.460
Vitamin C (mg)	68	25	39	73	87	225	83	31	60	77	97	276	0.579[3]
Vitamin A (μg)	709	182	517	793	847	177	634	294	409	614	761	159	0.577
Vitamin D (μg)	2.3	1.3	1.4	1.9	2.8	32	2.1	1.8	0.7	1.4	3.1	30	0.368[3]
Calcium (mg)	838	211	656	911	1002	240	721	260	513	606	933	206	0.349
Phosphorus (mg)	936	237	701	972	1097	347	815	177	663	744	981	302	0.229

Nutrient /day	<6 months (n=6)						≥6 months (n=13)						P
	Mean	SD	25th Centile	Median	75th centile	Mean as %RNI	Mean	SD	25th centile	Median	75th centile	Mean as %RNI	
Magnesium (mg)	175	37	151	168	196	206	201	53	165	188	230	237	0.323[3]
Sodium (mg)	1431	514	1074	1288	1849	286	1108	237	946	1155	1287	222	0.244[3]
Potassium (mg)	1974	461	1673	2060	2292	247	2087	401	1824	2118	2397	261	0.592
Chloride (mg)	2223	777	1688	2085	2656	278	1821	310	1534	1902	2018	228	0.244[3]
Iron (mg)	6.2	1.5	4.8	6.2	7.8	90	6.8	1.7	5.0	6.9	8.3	98	0.476
Zinc (mg)	6.4	1.7	5.3	5.6	7.9	129	5.6	0.8	5.2	5.5	6.0	112	0.289
Copper (mg)	0.7	0.2	0.5	0.6	0.8	164	0.8	0.3	0.7	0.8	1.0	211	0.106[3]
Selenium (µg)	26	4	22	25	28	170	26	12	19	23	33	176	0.521[3]
Iodine (µg)	176	67	111	177	236	252	141	62	90	113	193	202	0.210[3]

[1] For energy intakes in boys n=4 before 6 months, n=7 after 6 months

[2] For energy intakes in girls n=2 before 6 months, n=6 after 6 months

[3]Mann Whitney test

Abbreviations: CHO: carbohydrate, NSP: Non-starch polysaccharides, MUFA: Mono-unsaturated fatty acids, PUFA: Poly-unsaturated fatty acids, equiv: equivalents

3.3.4.9 Nutrient intakes at 18 months by method of complementary feeding

Looking at mean nutrient intakes stratified by method of feeding (Table 3-14), infants who followed baby-led weaning (n=7) had significantly higher intakes of selenium (p=0.028) than those who were spoon-fed (n=12). However the BLW group had significantly lower calcium (p=0.045) and vitamin A intakes (p=0.018) than the spoon-fed group at 18 months. Iodine intakes were also lower in the BLW group and were close to reaching statistical significance. The majority of mean nutrients were above the recommended intakes in both groups. Iron intakes were lower in the baby-led group (94% of RNI versus 97% for the spoon-fed group) however the difference did not reach statistical significance (p=0.840). The nutrient with the lowest %RNI remained vitamin D (28% in the baby-led group and 32% in the spoon-fed group) but the differences were not statistically significant.

Table 3-14 Average daily intake and distributions across 25^{th} -75^{th} centiles of energy, macronutrients, vitamins and minerals, stratified by method of complementary feeding at 18 months

	BLW (n=7)						Spoon-fed (n=12)						
Nutrient /day	**Mean**	**SD**	**25^{th} Centile**	**Median**	**75^{th} centile**	**Mean as %RNI**	**Mean**	**SD**	**25^{th} centile**	**Median**	**75^{th} centile**	**Mean as %RNI**	**P**
Energy KJ (boys) [1]	5305	1095	4258	5293	6364	143	5154	1146	3915	5269	5681	139	0.836
Energy kcal (boys) [1]	1262	264	1011	1257	1517	143	1226	271	933	1252	1350	139	0.838
Energy KJ (girls)[2]	4788	1280	3856	4260	-	139	4863	933	4251	4438	5687	141	0.926
Energy kcal (girls)[2]	1136	309	910	1010	-	138	1157	223	1011	1055	1355	140	0.911
Protein (g)	38.7	4.9	35.5	38.1	42.1	267	44.2	10.1	39.2	42.0	49.3	305	0.196[3]
Total fat (g)	49	10	41	46	56		52	7	47	50	58		0.358
Saturated Fats (g)	19	4	16	18	20		22	6	20	23	26		0.190
MUFA(g) (g)	17	5	14	15	20		16	4	13	16	19		0.944
PUFA(g)	8	3	4	8	11		8	4	5	7	10		0.910
Total CHO (g)	162	24	148	169	185		147	19	134	142	161		0.150
Total NSP	11	3	9	11	12		12	3	9	11	14		0.650[3]
Thiamine (mg)	0.9	0.2	0.7	0.9	1.1	193	1.0	0.2	0.8	1.0	1.2	210	0.192

Nutrient /day	BLW (n=7)						Spoon-fed (n=12)						
	Mean	SD	25th Centile	Median	75th centile	Mean as %RNI	Mean	SD	25th centile	Median	75th centile	Mean as %RNI	P
Riboflavin (mg)	1.1	0.4	0.7	1.0	1.2	182	1.4	0.4	1.0	1.6	1.8	241	0.090
Niacin (mg)	9.3	1.8	8.2	9.2	10.1	122	8.5	2.4	6.6	7.8	9.5	110	0.435
Pyridoxine B6 (mg)	1.2	.3	1.1	1.3	1.4	205	1.2	.2	1.1	1.2	1.3	190	0.912
B12 (µg)	2.4	1.3	1.7	1.8	2.4	478	3.3	1.4	2.4	4.0	4.4	670	0.155
Folate (µg)	183	33	155	190	213	262	179	43	148	178	211	255	0.810
Vitamin C (mg)	77	17	59	77	93	256	79	35	57	75	96	262	0.967[3]
Vitamin A (µg)	480	200	307	431	734	120	762	240	567	755	853	190	**0.018**
Vitamin D (µg)	2.0	1.7	0.9	1.8	2.6	28	2.2	1.7	1.0	1.7	3.2	32	0.773[3]
Calcium (mg)	612	172	506	534	800	175	843	248	584	911	997	241	**0.045**
Phosphorus (mg)	745	151	643	733	874	276	916	202	705	958	1030	339	0.071
Magnesium (mg)	185	37	156	174	220	217	198	56	159	179	232	233	0.711[3]
Sodium (mg)	1172	275	1009	1272	1412	234	1232	422	921	1170	1351	246	0.711[3]
Potassium (mg)	1937	402	1467	2059	2226	242	2119	419	1865	2154	2453	265	0.367
Chloride (mg)	1916	327	1817	1999	2166	240	1966	617	1477	1897	2189	246	0.650[3]
Iron (mg)	6.5	1.1	5.1	6.9	7.3	94	6.7	1.9	5.0	6.2	8.4	97	0.840

Nutrient /day	BLW (n=7)						Spoon-fed (n=12)						
	Mean	**SD**	**25th Centile**	**Median**	**75th centile**	**Mean as %RNI**	**Mean**	**SD**	**25th centile**	**Median**	**75th centile**	**Mean as %RNI**	**P**
Zinc (mg)	5.3	0.7	4.9	5.4	5.8	106	6.2	1.3	5.3	5.6	7.0	123	0.135
Copper (mg)	0.8	0.2	0.7	0.8	1.0	199	0.8	0.3	0.6	0.7	0.9	195	0.592[3]
Selenium (µg)	27	11	18	23	36	180	26	9	21	23	27	171	**0.028[3]**
Iodine (µg)	115	58	83	96	113	164	174	59	120	180	221	248	0.050

[1] For energy intakes in boys n=4 in BLW group, n=7 in spoon-fed group

[2] For energy intakes in girls n=3 in BLW group, n=5 in spoon-fed group

[3] Mann Whitney test

Abbreviations: CHO: carbohydrate, NSP: Non-starch polysaccharides, MUFA: Mono-unsaturated fatty acids, PUFA: Poly-unsaturated fatty acids, equiv: equivalents

3.3.4.10 Nutrient comparisons with DRVs at 18 months

At 18 months one boy, who was introduced to solids after 6 months and followed baby-led weaning had energy intakes below the estimated average requirements. In terms of vitamins, all infants had intakes below the RNI for vitamin D, 36.8% for niacin, 21.1% for Vitamin A and 5.3% for vitamins B6 and Thiamine respectively. Niacin was the only nutrient at 18 months where infants had intakes below the LRNI (10.5% of infants). In terms of mineral intakes, iron fared the worst again with just over half of infants below the RNI (57.9%) but unlike the 8 month time point none had intakes below the LRNI. Zinc intakes had improved since the 8 month point as 36.8% had intakes below the RNI, followed by 10.5% copper and 5.3% with intakes below the RNI for iodine and selenium. The proportion of infants with intakes below the RNI for vitamin A was significantly higher in the baby-led weaning group (p=0.009). There were no statistically significant differences in the proportion of infants below the RNI for all the remaining nutrients in any of the groups examined (Table 3-15).

Table 3-15 Proportion of participants (n) with average daily intake of energy, macronutrients, vitamins and minerals below the Reference Nutrient in take (RNI) (EAR for energy) across the sample and stratified by age of solids introduction and mode of complementary feeding at 18 months

		Age of Solids Introduction			**Mode of Complementary Feeding**		
Nutrient /day*	**All (n=19)**	<6 months (n=6)	≥6 months (n=13)	**P**	BLW (n=7)	Spoon-fed (n=12)	**P**
Energy (kcal) (boys)	1 (9.1)	0	1 (14.3)	1	0	1 (14.3)	1
Thiamine (mg)	1 (5.3)	0	1 (7.7)	1	0	1 (8.3)	1
Niacin (mg)	7 (36.8)	3 (50)	4 (30.8)	0.617	2 (28.6)	5 (41.7)	0.656
Pyridoxine B6 (mg)	1 (5.3)	0	1 (7.7)	1	0	1 (8.3)	1
Vitamin A (µg)	4 (21.1)	0	4 (30.8)	0.255	4 (57.1)	0	**0.009**
Vitamin D (µg)	19 (100)	6 (100)	13 (100)	-	7 (100)	12 (100)	-
Iron (mg)	11 (57.9)	4 (66.7)	7 (53.8)	1	4 (57.1)	7 (58.3)	1
Zinc (mg)	7 (36.8)	1 (16.7)	6 (46.2)	0.333	4 (57.1)	3 (25)	0.326
Copper (mg)	2 (10.5)	2 (33.3)	0	0.088	0	2 (16.7)	0.509
Selenium (µg)	1 (5.3)	0	1 (7.7)	1	1 (14.3)	0	0.368
Iodine (µg)	1 (5.3)	0	1 (7.7)	1	1 (14.3)	0	0.368

*All variables n (%)

[1] For energy intakes in boys n=4 in BLW group, n=7 in spoon-fed group, n=4 before 6 months, n=7 after 6 months

3.3.5 Iron Status

3.3.5.1 Iron status at 8 months

Fifteen mothers (75%) consented for a blood sample to be taken from their infants at 8 months. The reason given by the remaining mothers for not consenting was that they did not want to cause their infants distress by having a heel-prick without a medical reason. Fourteen samples were obtained as one infant was unwell on the day of blood collection. Haemoglobin (Hb) levels were found to be normally distributed and are shown in Table 3-16. Two infants had Hb levels below the WHO cut-off point for anaemia (106 and 108 g/l respectively) both of whom were male and introduced to solids after 6 months. One of them was following baby-led weaning. Using the ALSPAC and SACN cut-off points, no infants were classed as having a low haemoglobin level. No significant association was found between the age of first solids introduction and haemoglobin at the 8 month time point (p=0.951). There were no differences between any of the groups and haemoglobin levels or proportion of infants below any of the cut-off points at 8 months.

Table 3-16 Haemoglobin levels at 8 months across the whole sample and stratified by age of solids introduction and mode of complementary feeding

		Age of Solids Introduction			Mode of Complementary Feeding		
Haemoglobin (g/l)	**All (n=14)**	<6 months (n=4)	≥6 months (n=10)	**P**	BLW (n=6)	Spoon-fed (n=8)	**P**
Mean	116.8	117	116.7	0.951	115.8	117.5	0.708
Median	116	116	115.5		114	116	
SD	7.8	6.8	8.5		8.5	7.7	
Upper 95% CI	121.3	127.9	122.8		124.7	124	
Lower 95% CI	112.3	106.1	110.6		107	111	
n(%) <SACN cut-off[1]	0	0	0 (0)		0	0	
n(%) <WHO cut-off[2]	2 (14)	0	2 (20)	1	1 (16.7)	1 (12.5)	1
n (%) <ALSPAC cut-off[3]	0	0	0		0	0	

[1] Haemoglobin: 7-9 M <100g/L Scientific Advisory Committee on Nutrition (SACN). Iron and Health

[2] Haemoglobin: 1-5years <110g/L World Health Organization (WHO). Iron Deficiency Anaemia. Assessment, Prevention and Control. A guide for programme managers. 2001. Geneva: WHO, 2001.

[3]Haemoglobin: 8 M <97g/l (Emond et al. 1996)

3.3.5.2 Dietary iron intakes at 8 months

At 8 months, the mean dietary intake of iron was 3.25mg/day, with 70% of infants below the lowest reference nutrient intake (LRNI) for iron for that age group of 4.2mg/day and none of them achieving the recommended nutrient intake (RNI) of 7.8mg/day (Committee on Medical Aspects of Food Policy (COMA) 1991). Most of the iron derived from non-haem sources (3.35mg/day of non-haem iron as opposed to 0.09mg/day of haem iron). There were no significant differences between any of the groups of infants in total, haem and non-haem iron intakes (Table 3-17).

Table 3-17 Dietary iron intakes at 8 months across the whole sample and stratified by age of solids introduction and mode of complementary feeding

		Age of Solids Introduction			Mode of Complementary Feeding		
Dietary iron intakes (mg/day)	**All (n=20)**	<6 months (n=7)	≥6 months (n=13)	**P**	BLW (n=7)	Spoon-fed (n=13)	**P**
Total Iron							
Mean	3.25	3.66	3.02	0.317	2.99	3.38	0.545
Median	3.03	3.07	3.00		3.07	3.00	
SD	1.31	1.42	1.24		0.98	1.48	
Upper 95% CI	3.86	4.97	3.78		3.90	4.27	
Lower 95% CI	2.63	2.35	2.27		2.09	2.49	
n (%) below LRNI	14 (70)	5 (71.4)	9 (69.2)	1	6 (85.7)	8 (61.5)	0.354
n (%) below EAR	20 (100)	7 (100)	13 (100)		7 (100)	13 (100)	
n (%) below RNI	20 (100)	7 (100)	13 (100)		7 (100)	13 (100)	
Haem iron							
Mean	0.05	0.09	0.03	0.351	0.05	0.06	0.438

Dietary iron intakes (mg/day)	All (n=20)	Age of Solids Introduction <6 months (n=7)	≥6 months (n=13)	P	Mode of Complementary Feeding BLW (n=7)	Spoon-fed (n=13)	P
Median	0.03	0.08	0.02		0.04	0.02	
SD	0.07	0.09	0.03		0.04	0.08	
Upper 95% CI	0.08	0.18	0.05		0.08	0.1	
Lower 95% CI	0.02	0.007	0.01		00.1	0.009	
Non-haem iron							
Mean	3.19	3.56	2.99	0.366	2.95	3.32	0.553
Median	2.92	2.83	3.02		3.05	2.83	
SD	1.30	1.44	1.24		0.98	1.48	
Upper 95% CI	3.81	4.58	3.74		3.86	4.22	
Lower 95% CI	2.60	2.13	2.24		20.4	2.43	

COMA dietary reference values cut-off points for iron (RNI: reference nutrient intake of 7.8mg/day, EAR: estimated average requirement of 6mg/day, LRNI: Lower reference nutrient of 4.2mg/day)

3.3.5.3 Contribution of food groups to iron intakes at 8 months

Formula milk was a major contributor to iron intake, accounting for 43.7% of dietary iron among the infants who consumed it (n=8), and 17.5% across the whole sample. The same was true for commercial infant foods which provided 22.2% of iron among consumers and 7.8% overall. Breast milk provided, on average, 0.17 mg of iron per day or 5.3% of the iron intake across the sample. The contribution of formula and breast milk to individual iron intakes versus all the other food sources in the infant's diet is presented in Figure 3-3. Contributions of non-infant-specific food groups to iron intakes include: cereal and cereal dishes 21.3% across the sample and in particular breakfast cereals - 24.1% among 11 consumers and 13.3% overall; eggs and egg dishes (21.5% among 10 consumers, 10.8% overall) and pulses/lentils (19.7% among 5 consumers, 4.9% overall). Meat (including red, white and processed meat, sausages, burgers and offal) contributed 9.8% of iron intakes among 12 consumers (5.8% overall). Haem iron was provided by meat, fish and commercial infant foods. Aggregated food groups and their individual contribution to iron intakes are shown in detail in Table 3-18.

Figure 3-3 Individual (n=20) mean iron intake (mean of 3 days) from formula milk, breast milk and all other sources and the COMA dietary reference values cut-off points for iron

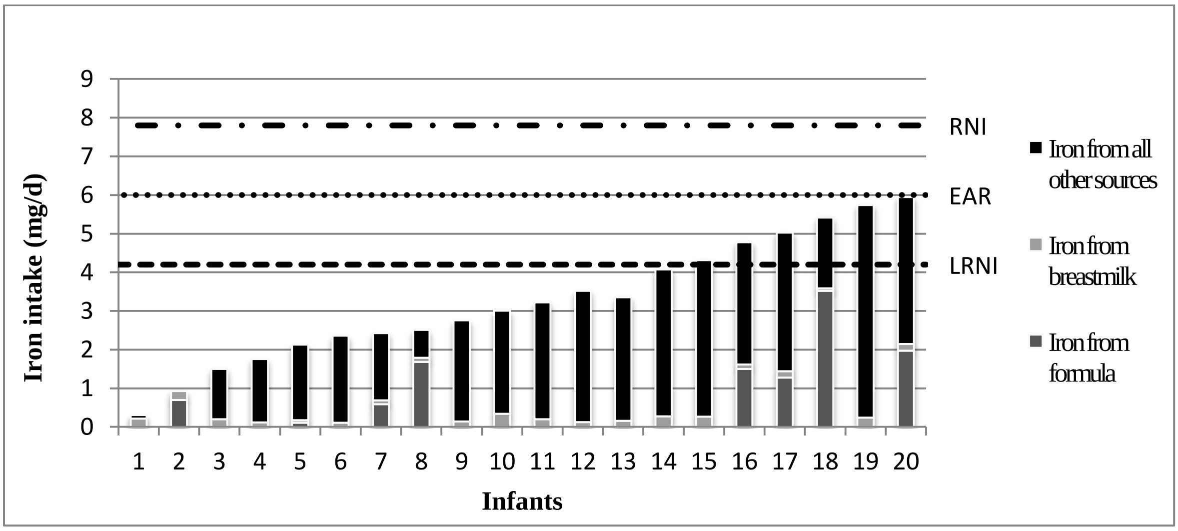


Table 3-18 Contribution of food groups (food sources) to daily iron intake (mg), at 8 months

Food group[a]	Contribution to daily iron intakes			
	% Fe	**Total Fe (mg)**	**Haem Fe (mg)**	**Non-haem Fe (mg)**
Non-infant specific foods:				
Cereals and cereal dishes	21.3	0.69	0	0.69
Consumers (n=20)				
Milk and milk products				
Consumers (n=18)	3.1	0.1	0	0.1
All (n=20)	2.8	0.09	0	0.09
Meat and meat products				
Consumers (n=12)	9.8	0.32	0.12	0.2
All (n=20)	5.8	0.19	0.07	0.12
Fish and fish dishes				
Consumers (n=6)	5.2	0.17	0.06	0.11
All (n=20)	1.5	0.05	0.02	0.03
Eggs and egg dishes				
Consumers (n=10)	21.5	0.7	0	0.7
All (n=20)	10.8	0.35	0	0.35
Vegetables and potatoes				
Consumers (n=19)	14.5	0.47	0	0.47
All (n=20)	13.8	0.45	0	0.45

Food group[a]	Contribution to daily iron intakes			
	% Fe	Total Fe (mg)	Haem Fe (mg)	Non-haem Fe (mg)
Lentils and pulses				
Consumers (n=5)	19.7	0.64	0	0.64
All (n=20)	4.9	0.16	0	0.46
Infant formula				
Consumers (n=8)	43.7	1.42	0	1.42
All (n=20)	17.5	0.57	0	0.57
Of which:				
'First milk'				
Consumers (n=1)	39.4	1.28	0	1.28
All (n=20)	1.8	0.06	0	0.06
Follow-on milk				
Consumers (n=7)	44.3	1.44	0	1.44
All (n=20)	15.4	0.5	0	0.5
Breast milk[b]				
Consumers (n=20)	5.2	0.17	0	0.17
Commercial infant foods:				
Consumers (n=7)	22.2	0.72	0.04	0.68
All (n=20)	7.8	0.25	0.01	0.24
of which:				
Meat and fish based products and dishes				
Consumers (n=3)	19.7	0.64	0.08	0.57
All (n=20)	3.1	0.1	0.01	0.09

Food group[a]	Contribution to daily iron intakes			
	% Fe	Total Fe (mg)	Haem Fe (mg)	Non-haem Fe (mg)
Vegetable based foods and dishes				
Consumers (n=3)	30.2	0.98	0.03	0.96
All (n=20)	3.1	0.1	0	0.1
Deserts				
Consumers (n=4)	7.1	0.23	0	0.23
All (n=20)	1.5	0.05	0	0.05
Cereal based foods and dishes				
Consumers (n=1)	42.8	1.39	0	1.39
All (n=20)	2.2	0.07	0	0.07

[a] Some food groups are not included due to small numbers of consumers; e.g. nuts and seeds and savoury snacks

[b] Typical volumes of breast milk for full feeds were estimated from MRC data (Paul et al. 1988) to be approximately 100g for those aged 8 months. It was assumed that a feed of 10 minutes or longer was equivalent to a full feed. For feeds of less than 10 minutes duration weights were calculated proportionately at at 10g/min for 8 months.

3.3.5.4 Nutrients relevant to iron status at 8 months

Table 3-19 describes the correlations between nutrient intakes and haemoglobin at 8 months. None of the nutrients were associated with haemoglobin levels at this time point.

Table 3-19 Pearson correlations between selected iron enhancers and inhibitors and haemoglobin levels at 8 months and partial correlations adjusted for energy

	Haemoglobin (n=14)			
	Unadjusted		**Energy Adjusted**	
Nutrients	Correlation coefficient	**P**	Correlation coefficient	**P**
Energy	0.231	0.427	n/a	n/a
Iron	-0.074	0.801	-0.384	0.195
Haem iron*	0.056	0.863	-0.004	0.990
Vitamin C*	0.302	0.294	0.201	0.509
Calcium	0.103	0.725	-0.162	0.597
NSP	0.000	0.999	-0.408	0.166

For haem iron n=12 due to zero intakes on 2 occasions

*variables appropriately transformed to normalise distributions

3.3.5.5 Iron status at 18 months

Haemoglobin (Hb) levels were found to be normally distributed at 18 months and are shown in Table 3.20.. One infant (female) had Hb levels below the WHO cut-off point (105g/l). She had been introduced to solids before 6 months and did not follow a baby-led weaning approach. Using the ALSPAC cut-off point, no infants were classed as having a low haemoglobin level. No significant association was found between the age of first solids introduction and haemoglobin at the 18 month time point (p=0.795). There were no differences between any of the groups and haemoglobin levels or proportion of infants below any of the cut-off points at 18 months.

Table 3-20 Haemoglobin levels at 18 months across the whole sample and stratified by age of solids introduction and mode of complementary feeding

		Age of Solids Introduction			Mode of Complementary Feeding		
Haemoglobin (g/l)	**All (n=13)**	<6 months (n=4)	≥6 months (n=9)	**P**	BLW (n=5)	Spoon-fed (n=8)	**P**
Mean	120.8	121.5	120.6	0.861	118	122.6	0.356
Median	121	123.5	121		119	125.5	
SD	0.84	12.6	6.8		4.4	10	
Upper 95% CI	125.9	101.4	115.4		112.6	114.2	
Lower 95% CI	115.8	141.6	125.8		123.4	131	
n (%) < ALSPAC cut-off[1]	0	0	0		0	0	
n (%) <WHO cut-off[2]	1 (7.7)	1 (25)	0	0.316	0	1(12.5)	1

[1] Haemoglobin: 12-18mo <100g/L (Sherriff et al., 1999)

[2] Haemoglobin: 1-5years <110g/L World Health Organization (WHO). Iron Deficiency Anaemia. Assessment, Prevention and Control. A guide for programme managers. 2001. Geneva: WHO, 2001.

3.3.5.6 Dietary iron intakes at 18 months

Mean iron intakes at 18 months were 6.7mg/day which were close to the 6.9mg/day reference nutrient intake set by COMA (Committee on Medical Aspects of Food Policy (COMA) 1991) although over half of the sample was below the RNI. No infants were below the LRNI reference (3.7 mg/day) and a fifth of the sample had iron intakes below the EAR reference (5.3mg/day). Non-haem sources provided the majority of iron intake at this time point. There were no significant differences between any of the groups of infants in total, haem and non-haem iron intakes (Table 3-21).

Table 3-21 Dietary iron intakes at 18 months across the whole sample and stratified by age of solids introduction and mode of complementary feeding

		Age of Solids Introduction			Mode of Complementary Feeding		
Dietary iron intakes (mg/day)	**All (n=19)**	<6 months (n=6)	≥6 months (n=13)	**P**	BLW (n=7)	Spoon-fed (n=12)	**P**
Total Iron							
Mean	6.61	6.21	6.79	0.476	6.51	6.67	0.840
Median	6.28	6.21	6.93		6.93	6.21	
SD	1.61	1.48	1.69		1.13	1.88	
Upper 95% CI	7.38	7.76	7.81		7.56	7.86	
Lower 95% CI	5.83	4.65	5.77		5.46	5.47	
n (%) below LRNI	0	0	0		0	0	
n (%) below EAR	4 (21.1)	2 (50)	2 (50)	0.557	0	4 (100)	0.245
n (%) below RNI	11 (57.9)	4 (66.7)	7 (53.8)	1	4 (57.1)	7 (58.3)	1
Haem iron							
Mean	0.11	0.12	0.10	0.579	0.15	0.08	0.340
Median	0.09	0.11	0.09		0.10	0.08	
SD	0.12	0.09	0.13		0.15	0.09	

Dietary iron intakes (mg/day)	All (n=19)	Age of Solids Introduction			Mode of Complementary Feeding		
		<6 months (n=6)	≥6 months (n=13)	**P**	BLW (n=7)	Spoon-fed (n=12)	**P**
Upper 95% CI	0.17	0.21	0.18		0.30	0.14	
Lower 95% CI	0.05	0.03	0.02		0.01	0.03	
Non-haem iron							
Mean	6.50	6.08	6.69	0.478	6.35	6.58	0.782
Median	6.27	6.08	6.90		6.90	6.08	
SD	1.67	1.48	1.78		1.25	1.92	
Upper 95% CI	7.30	7.63	7.76		7.51	7.81	
Lower 95% CI	5.69	4.53	5.62		5.20	5.36	

COMA dietary reference values cut-off points for iron (RNI: reference nutrient intake of 6.9mg/day, EAR: estimated average requirement of 5.3mg/day, LRNI: Lower reference nutrient of 3.7mg/day)

3.3.5.7 Contribution of food groups to iron intakes at 18 months

Only one infant was consuming formula milk at 18 months and 3 infants were having commercial infant foods, therefore the majority of dietary iron at this time point derived from family foods. Cereal and cereal dishes were the major contributors to iron intake, accounting for 38.4% of dietary iron across the whole sample. Fruit, vegetables and lentils were the other main contributors with meat contributing 5.8% of iron across the whole sample (8.5% among 9 consumers).

Aggregated food groups and their individual contribution to iron intakes are shown in detail in Table 3-22.

Table 3-22 Contribution of food groups (food sources) to daily iron intake (mg), at 18 months

	Contribution to daily iron intakes			
Food group[a]	**% Fe**	**Total Fe (mg)**	**Haem Fe (mg)**	**Non-haem Fe (mg)**
Non-infant specific foods				
Cereals / cereal dishes				
Consumers (n=19)	38.4	2.57	0	2.57
Fruit				
Consumers (n=19)	13.3	0.89	0	0.89
Milk / milk products				
Consumers (n=19)	5.4	0.36	0	0.36
Meat / meat products				
Consumers (n=9)	8.5	0.57	0.17	0.41

Food group[a]	Contribution to daily iron intakes			
	% Fe	Total Fe (mg)	Haem Fe (mg)	Non-haem Fe (mg)
All (n=19)	5.8	0.39	0.12	0.28
Fish and fish dishes				
Consumers (n=11)	4.6	0.31	0.05	0.26
All (n=19)	2.7	0.18	0.03	0.15
Eggs and egg dishes				
Consumers (n=11)	14.6	0.98	0	0.98
All (n=19)	8.5	0.57	0	0.57
Vegetables and potatoes				
Consumers (n=18)	14	0.94	0	0.94
All (n=19)	13.3	0.89	0	0.89
Lentils and pulses				
Consumers (n=11)	15.2	1.02	0	1.02
All (n=19)	8.8	0.59	0	0.59
Savoury snacks				
Consumers (n=15)	4.2	0.28	0	0.28
All (n=19)	3.3	0.22	0	0.22
Peanuts / peanut butter				
Consumers (n=9)	7.8	0.52	0	0.52
All (n=19)	3.7	0.25	0	0.25
Breast milk[b]				

Food group[a]	Contribution to daily iron intakes			
	% Fe	Total Fe (mg)	Haem Fe (mg)	Non-haem Fe (mg)
Consumers (n=9)	4.3	0.29	0	0.29
All (n=19)	2.1	0.14	0	0.14

[a] Some food groups are not included due to small numbers of consumers; e.g. commercial infant foods, formula

[b] Typical volumes of breast milk for full feeds were estimated from MRC data (Paul et al. 1988) to be approximately 100g for those aged 8 months. It was assumed that a feed of 10 minutes or longer was equivalent to a full feed. For feeds of less than 10 minutes duration weights were calculated proportionately at at 10g/min for 8 months.

3.3.5.8 Nutrients relevant to iron status at 18 months

Table 3-23 describes the correlations between nutrient intakes and haemoglobin at 18 months. None of the nutrients were associated with haemoglobin at this time point.

Table 3-23 Pearson Correlations between selected iron enhancers and inhibitors and haemoglobin levels at 18 months and partial correlations adjusted for energy

	Haemoglobin (n=13)			
	Unadjusted		Energy Adjusted	
Nutrients	Correlation coefficient	P	Correlation coefficient	P
Energy	0.440	0.133	n/a	n/a
Iron	0.332	0.268	0.331	0.294
Haem iron*	0.025	0.935	-0.300	0.344
Vitamin C*	0.404	0.171	0.222	0.488
Calcium	0.101	0.742	-0.120	0.711
NSP	0.499	0.082	0.402	0.195

*variables appropriately transformed to normalise distributions

3.3.6 Eating behaviours

3.3.6.1 Feeding practices by 8 months

An overview of the timings of introduction of solids with different textures is given in Table 3-24.

One infant (5%) was introduced to purees at 4 months, one at 4.5 months, 2 infants (10%) at 5 months and 3 (15%) at 5.5 months. Six mothers (30%) reported that they did not offer pureed foods to their infants at all, as they were following baby-led weaning. None of the infants in the baby-led weaning group were introduced to solids before 6 months. The rest of the sample (7 infants, 35%) was introduced to pureed food at or after 6 months. Introduction of puree food was significantly earlier in infants introduced to solids before six months (p=0.01).

Only one infant from the early complementary feeding group was introduced to foods with a lumpy texture before six months. From the late complementary feeding group, one infant (7.7%) had not been introduced to lumpy solids by 8 months, whereas 2 mothers (28.6%) reported that due to following a baby-led weaning approach they did not introduce lumps at all. In both groups, all other infants were introduced to lumpy solids between 6-7 months. There were no significant differences with regards to introducing lumpy foods between early and late complementary feeding groups (p=0.692) nor BLW and spoon-fed groups (p=0.195).

By 8 months, all infants had been introduced to finger goods (range 6-8 months). Those who were introduced to solids before 6 months were offered finger foods later than the ones introduced to solids after 6 months; however the difference was not statistically significant (p=0.311). On the contrary, infants following baby-led weaning were introduced to finger foods significantly earlier than those who were spoon-fed (p=0.046).

The main reason given for introducing solids (either pureed, lumpy, or finger foods) was that the mother felt that the infant was ready for solids food (30%); reported signs of this included grabbing food (5%), teething (5%), smacking lips when other people were eating (5%), showing interest in food (5%), waking up at night (5%). Other reasons included being no longer satisfied with breast milk (15%) or advice from friends (5%). Three participants (15%) reported that they got advice from health professionals to introduce solids slightly earlier in order for the infant to have established a full eating routine by 6 months.

Table 3-24 Timing of introduction of different textures of solids foods across the whole sample and stratified by age of solids introduction and mode of complementary feeding

Food textures	All (n=20)	Age of Solids Introduction			Mode of Complementary Feeding		
		<6 months (n=7)	≥6 months (n=13)	**P**	BLW (n=7)	Spoon-fed (n=13)	**P**
Pureed solids	5.8 (5,6)	5 (4.5, 5.5)	6 (constant)	**0.01**	-	-	-
Lumpy solids	6 (6,7)	6 (6,7)	6 (6,7)	0.692	6.5 (6,7)	6 (Constant)	0.195
Finger foods	6.5 (6,7)	7 (6.5, 7)	6 (6,7)	0.311	7 (6.25, 7)	6 (6,6.5)	**0.046**

All variables: Median (IQR)

For Pureed solids n=14 due to BLW not introducing this texture (<6 months: n=7, ≥6 months: n=7)

For Lumpy solids n=17 due to some infants in the BLW group not introducing this texture (<6 months: n=7, ≥6 months: n=10, BLW: n=5, spoon-fed: n=12)

3.3.6.2 Eating behaviours at 8 months

Around the age of 8 months, mothers were asked about their infants' eating habits and behaviour since the introduction of the first solid foods. When asked if their infants had definite foods preferences, 80% of participants (16 mothers) responded that their infant would eat almost anything whereas 4 mothers (20%) considered their infants to be quite choosy. There were no significant differences in food preferences between early versus late introduction of solids (p=0.439) or baby-led weaning versus spoon-feeding (p= 0.148).

Four mothers (20%) reported having difficulty feeding solids to their infants, 8 (40%) reported occasional difficulty whereas 8 (40%) did not report any difficulty.

Mothers were also asked to comment on common feeding problems since introducing solids to their infants and how worried they were, when those occurred. The results are shown in Figure 3-4. Additional feeding problems reported were teething problems/illness that affected appetite, having a sensitive stomach, not tolerating some foods and mealtimes taking a long time to finish. There were also reports of conflicting weaning advice from health visitors that were confusing and created additional stress. There were no significant differences in the proportions of infants with reported feeding difficulties between the early and late complementary groups or between the baby-led weaning and spoon-feeding groups (Table 3.25).

Figure 3-4 Reported feeding problems at 8 months

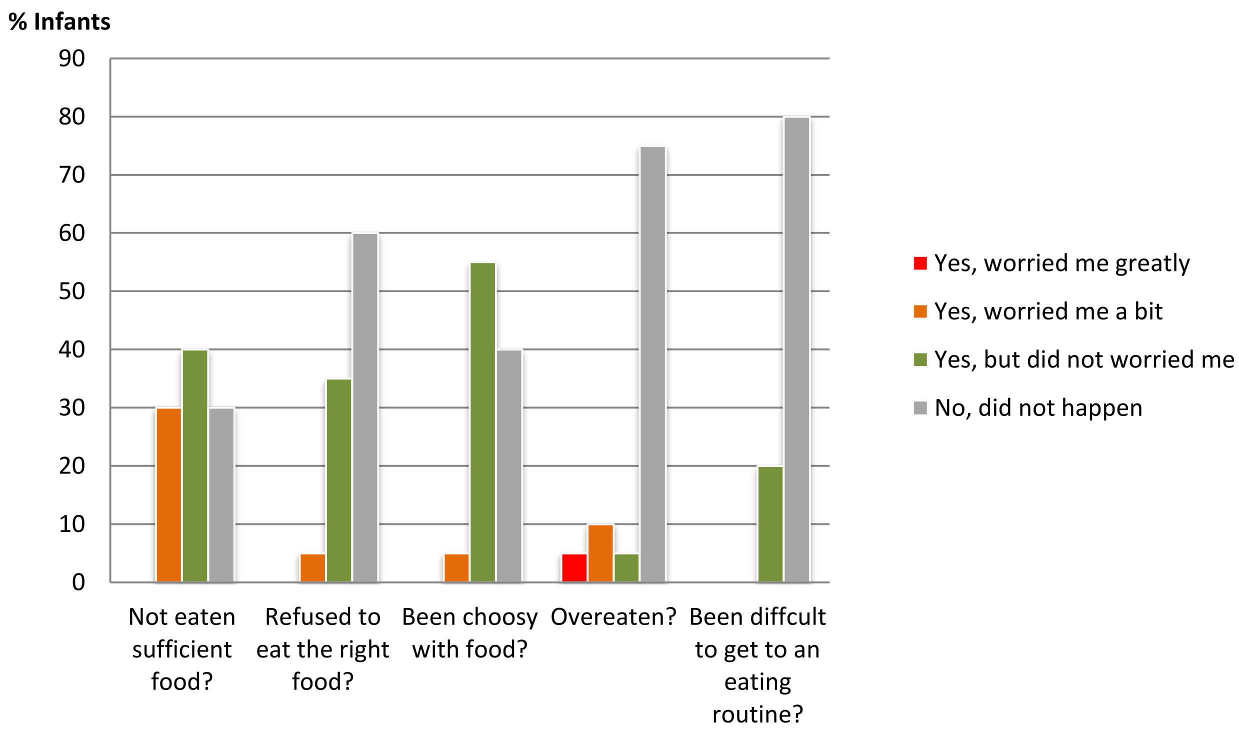


Table 3-25 Reported feeding difficulties at 8 months, across the whole sample and stratified by age of solids introduction and mode of complementary feeding

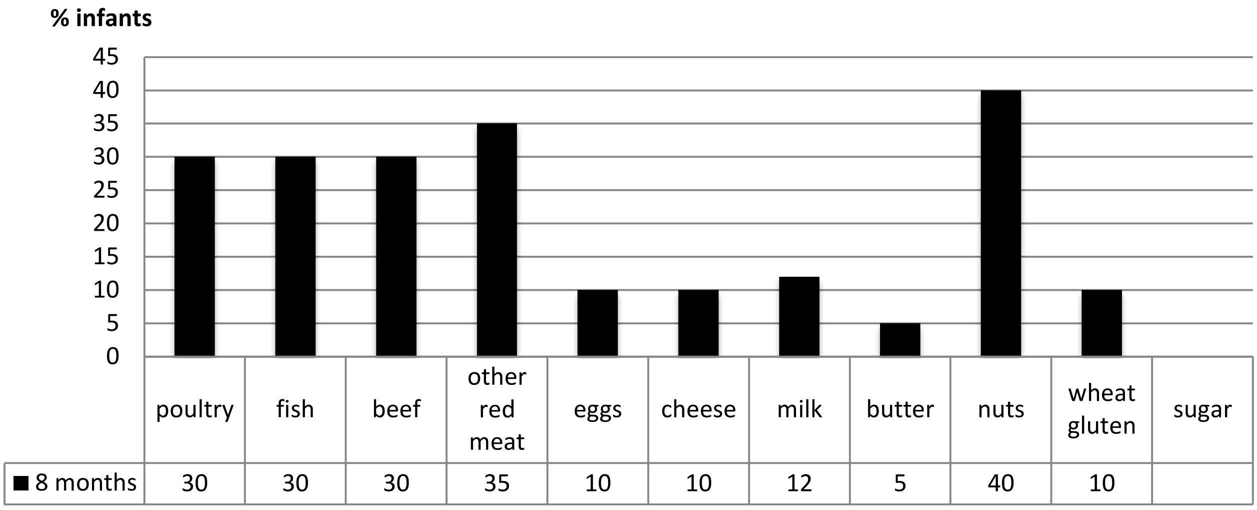
Eating behaviours	All (n=20)	Age of Solids Introduction			Mode of Complementary Feeding		
		<6 months (n=7)	≥6 months (n=13)	**P**	BLW (n=7)	Spoon-fed (n=13)	**P**
Any feeding difficulty	12 (60)	4 (57.1)	8 (61.5)	1	4 (57.1)	8 (61.5)	1
Insufficient amount of food	14 (70)	3 (42.9)	11 (84.6)	0.122	5 (71.4)	9 (69.2)	1
Refused to eat the right food	8 (40)	1 (14.3)	7 (53.8)	0.158	2 (28.6)	6 (46.2)	0.642
Been choosy with food	12 (60)	5 (71.4)	7 (53.8)	0.642	2 (28.6)	10 (76.9)	0.062
Overeaten	4 (21.1)	2 (28.6)	2 (16.7)	0.603	1 (16.7)	3 (23.1)	1
No feeding routine	4 (20)	0	4 (30.8)	0.249	1 (14.3)	3 (23.1)	1

For all variables: n (%)

3.3.6.3 Foods avoidance at 8 months

Thirteen participants (65%) reported that they actively avoided giving their infants certain foods. At 8 months, 30% (6) of mothers avoided feeding poultry, fish and beef, 35% (7) mothers avoided feeding any other type of red meat and 40% (8) avoided feeding nuts to their infants. (Figure 3-5). Other foods that some mothers avoided giving their infants included seafood, honey (5%) and tomatoes (5%). Three mothers (15%) had not yet introduced some foods to their infants including nuts, eggs and beef and other red meat. There were no differences in avoidance of any of the above foods between the early and late complementary feeding groups (data not shown). When comparing those who followed baby-led weaning with those who were spoon-fed, there were no significant differences in avoidance all foods apart from nuts where significantly more mothers from the BLW group were avoiding feeding them to their infants (p=0.015). None of the infants were on a special diet although one (5%) was reported not be able to eat dairy. When asked if mothers felt that their infant ate differently at 8 months of age compared to other children, 11 (55%) responded yes. Ten mothers explained the reasons why they thought that was. Of them seven (35%) listed baby-led weaning as the main difference, one mentioned that her infant could not cope with baby-led weaning as opposed to most of her peers. One mother used mostly commercial foods because of work commitments and one mother had excluded meat from the infant's diet. When stratified by age of solids introduction, 10 mothers (76.9%) who introduced solids to their infants at 6 months and 1 mother (14.3%) from who introduced solids to their infant before 6 months felt that their infants ate differently than their peers and that difference was significant (p=0.017). More mothers in the BLW group (6 mothers, 87.5%) felt their infant ate differently than others as opposed to 5 mothers (38.5%) in the spoon-fed group. Differences in maternal perception of different eating patterns, stratified by mode of complementary feeding were close to, but did not reach statistical significance (p=0.07).

Figure 3-5 Food avoidance at 8 months



3.3.6.4 Feeding practices by 18 months

Nine mothers (47.4%) were still breastfeeding their infants at 18 months. Of the rest of the sample, 2 (10.5%) stopped breastfeeding at 9 months, 1 (5.3%) at 10.5 months, 2 at 11 and 12 months respectively (10.5%) and 1 at 14, 15 and 16 months respectively (5.3% in each category). The mean age of infants when they stopped breastfeeding was 11.95 months (SD: 2.4). There were no statistically significant differences in the duration of breastfeeding between those introduced to solids before (10.9 months, SD: 1.4, n=4) or after 6 months (12.7 months SD: 2.7, n=6), (p= 0.268). The same was true between those who followed baby-led weaning (12 months, SD: 4.2, n=2) and those who were spoon-fed (11.9 months, SD: 2.2, n=8) (p=0.976).

3.3.6.5 Eating behaviours at 18 months

In terms of food preferences the majority of mothers (n=14, 73.7%) reported that their infant would eat almost anything. Five mothers (26.3%) reported that their infants were quite choosy. Twelve mothers (63.2%) said that their infant did not have any particular difficulties with feeding. 5 mothers (26.3%) reported occasional difficulty with feeding, whereas 2 mothers (10.5%) said their infant had some feeding difficulties. There were no differences between the early versus late complementary feeding groups in food preferences and feeding difficulties (p=1 for both). The same was true for the baby-led versus spoon-fed groups (p=0.603 for food preferences, p=0.804 for feeding difficulties).

Mothers were also asked to comment on common feeding problems that their infants were having at 18 months and how worried they were when those occurred. The results are shown in Figure 3-6. In summary, one mother (5.3%) worried greatly and two (10.6%) worried a bit about their infant not eating sufficient food, whereas 3 mothers (15.8%) worried a bit about their infants not eating the right food. Most mothers were not worried about any of the feeding problems (insufficient eating, not eating the right food, overeating, establishing an eating routine) at 18 months. There were no significant differences in the proportions of infants with reported feeding difficulties between the early and late complementary groups or between the baby-led weaning and spoon-feeding groups (Table 3-26).

Figure 3-6 Feeding problems at 18 months

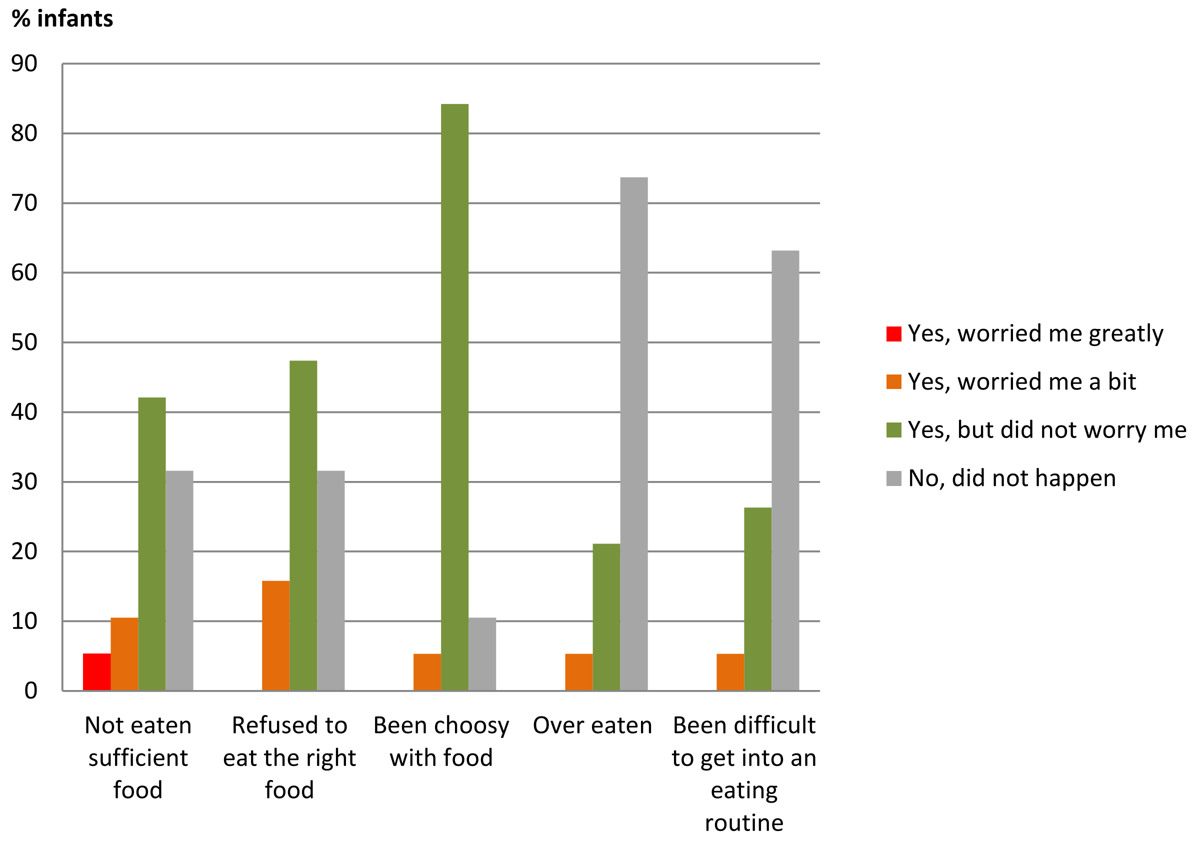


Table 3-26 Reported feeding difficulties at 18 months, across the whole sample and stratified by age of solids introduction and mode of complementary feeding

Eating Behaviours	All (n=19)	Age of Solids Introduction			Mode of Complementary Feeding		
		<6 months (n=6)	≥6 months (n=13)	P	BLW (n=7)	Spoon-fed (n=12)	P
Any feeding difficulty	7 (36.8)	2 (33.3)	5 (38.5)	1	2 (28.6)	5 (41.7)	0.656
Insufficient amount of food	11 (64.7)	3 (60)	8 (66.7)	1	4 (66.7)	7 (63.6)	1
Refused to eat the right food	12 (66.7)	3 (50)	9 (75)	0.344	3 (50)	9 (75)	0.344
Been choosy with food	17 (89.5)	6 (100)	11 (84.6)	1	5 (71.4)	12 (100)	0.123
Overeaten	5 (26.3)	1 (16.7)	4 (30.8)	0.480	2 (28.6)	3 (25)	1
No feeding routine	6 (33.3)	1 (16.7)	5 (41.7)	0.600	3 (50)	3 (25)	0.344

For all variables: n (%)

For Insufficient amount of food n=17 due to missing data (<6 months: n=5, ≥6 months: n=12) , BLW: n=6, spoon-fed: n=11)

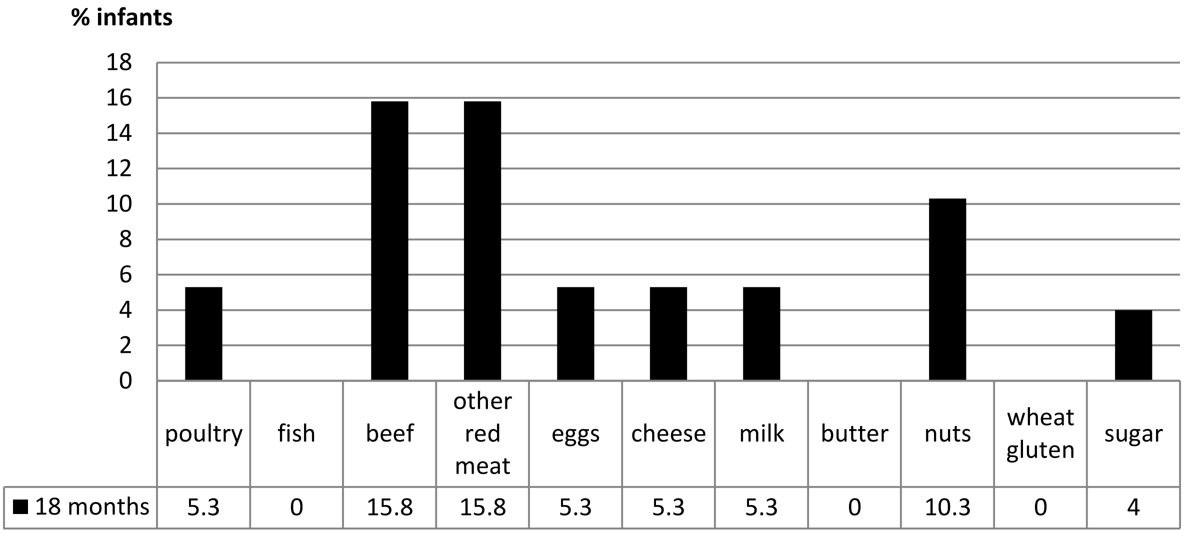
For Refused to eat the right food n=18 due to missing data (<6 months: n=6, ≥6 months: n=12) , BLW: n=6, spoon-fed: n=12)

3.3.6.6 Foods avoidance at 18 months

At 18 months, 1 mother avoided feeding poultry, eggs, cheese and milk (5.3%) to her infant, none of the mothers avoided feeding fish, wheat/gluten or butter, 3 avoided feeding beef and other red meat (15.8%), 2 avoided nuts (10.6%) and 4 avoided feeding sugar (21.1%) to their infants (Figure 3-7). There were no statistically significant differences in any of the foods avoided when the infants were stratified by age of solids introduction and by mode of complementary feeding (data not shown).

When asked if they thought their infants ate differently at 18 months from other infants of the same age, 7 mothers (36.8%) reported differences. However, the majority of the comments referred to their infants eating a wider variety of foods, more “adult” foods (not purees) and being less fussy than their peers. There were no statistically significant differences between infants who were introduced to solids before or after 6 months and mother’s reports of eating differently than their peers (p=1) nor between infants who followed baby-led weaning compared to those who were spoon-fed (p=0.332).

Figure 3-7 Food avoidance at 18 months



3.4 Discussion

The sample consisted of a small group of mostly working, non-smoking, well-educated mothers of a higher socioeconomic status, which is consistent with the characteristics of mothers who are likely to breastfeed for longer and introduce solids to their infants later as described in the infant feeding survey (McAndrew et al. 2012a). There were no statistically significant differences in the socio-demographic characteristics between infants introduced to solids before or after 6 months and between infants following baby weaning or those being spoon-fed. The only statistically significant difference between both groups was the age at which they were introduced to solids.

3.4.1 Main Findings

3.4.1.1 Growth

Growth of the sample as a whole was comparable to the WHO standards, demonstrating that, irrespective of age of solids introduction, weight, length and BMI-for-age at 8 and 18 months was similar to the standard in this group of breast-fed infants. A study in an exclusively breast-fed (for 6 months) population of 206 infants in Greece who were introduced to solids after six months and were followed up from birth to 36 months yielded similar results (Patsourou et al. 2012).

Mean birth weight in our study population was similar to a nationally representative sample: in the Millennium Cohort Study (n=16,157) unadjusted birth weight was 3.42 (95% CI: 3.40-3.43) kg (Kelly et al. 2009). A study by Fields (Fields et al. 2011) who followed up exclusively breast-fed infants and measured their body composition from birth to 6 months found a similar mean birth weight (3.46 +/- 0.39kg, n=160) , however there was high loss to follow up by 6 months mainly due to loss of exclusivity of breastfeeding (weight 7.13 +/- 0.73kg for girls n=35 and 7.40 +/- 0.97kg for boys n=30) resulting to similar sample size as our study. In a group of infants exclusively breast-fed for 6 months in Belarus (Kramer et al. 2003), infants were just over half a standard deviation heavier (weight-for-age z-score 0.53 versus -0.08) at 6 months and just under half a standard deviation heavier at the 8-9 months' time point (weight-for-age z-score 0.48 versus 0.08) than in our sample. In terms of length-for-age the Belarusian infants were slightly shorter than our sample at 8-9 months (z-score length-for-age -0.06 versus 0.12). However these values were adjusted for maternal education, number of siblings, anthropometrics at birth and 3 months and other parameters, whereas our sample was too small to allow such adjustments to be made. There could also be genetic differences between the two populations as well as cultural variations in terms of feeding practices. Also, birth weight alone is not a sensitive indicator of nutritional exposure in utero as it is influenced by additional parameters such as maternal phenotype, parity and habits (such as smoking) and other environmental factors (Scientific Advisory Committee on Nutrition 2011b). Although we had information on maternal smoking we were not able to adjust for it due to the small sample size.

At 8 months, those introduced to solids earlier were still lighter than the late complementary feeding group with a significant difference in BMI-for-age z-score, perhaps indicating a slower progression in the feeding process than those in the later complementary feeding group. It is also worth noting though that the early group was a centimetre longer which may have contributed to the lower BMI and weight-for-length z-score values. This is in line with data from the Euro-growth study (Haschke & van't Hof 2000),which found that fully breast-fed infants who were introduced to solids earlier (before 4-5 months) were taller and lighter, with lower BMI-for-age z-scores at 12 months compared to infants who were introduced to solids later.

By 18 months, infants introduced to solids before 6 months were still lighter than the later complementary feeding group, however the difference between the 2 groups remained non-significant as well as all other growth indices at 18 months. This finding is in contrast with other findings from the literature; Baker and colleagues followed predominantly breast-fed infants and found that infants who started solids early had a significantly higher weight gain rate in the first year than those who started solids later (224.2g additional weight, $p < 0.0001$). A possible explanation could be that both groups started solids later than the cut-offs used in other studies, and therefore the difference in timing of solids introduction although significant, was not large enough in practical terms between the 2 groups.

Similarly to the age of solids introduction groups the birth weights were almost identical for both groups (those who followed baby-led weaning versus those who were spoon-fed). At 8 months, BLW infants were significantly heavier than the spoon-fed group, although in the latter, infants were about 1cm longer. This could provide a possible explanation for the significant differences in weight-for-length and BMI-for-age z-scores. Unfortunately we did not have length measurements at 6 months to see if that was the case at that time point. At 18 months BLW infants were still significantly heavier than the spoon-fed group. In contrast, lower BMI in BLW infants in later infancy was observed in a study by Townsend and Pitchford (Townsend & Pitchford 2012) the only study to our knowledge which examined the impact of BLW on food preferences and health related outcomes including BMI. Parents of 155 children (20-78 months) completed a food preference questionnaire. As mentioned previously, the baby-led weaning group self-reported height and weight, whereas the spoon-fed group was measured in a study laboratory. BLW was self-reported, but was verified by interrogating responses to items related to weaning methods. The BLW group had lower BMI; mean percentile BMI rank was close to expected average (50th), whereas the spoon-fed group had a higher BMI centile rank and was more likely to be classed as overweight. BMI z-scores were significantly different between the two groups. There were more toddlers classed as obese (z more than 2) in spoon-fed group (n=8) as opposed to the BLW group (n=1). However, significantly more toddlers were classed as underweight (z-scores at or above -2) in the BLW category (Fisher's exact p= 0.02). Toddlers in the BLW group had a lower BMI that was not accounted by differences in birth weight, parental BMI and socioeconomic status. The authors acknowledged the high proportion of missing BMI data in the BLW group (32%). There could also be potential recall bias as the information on age of solids introduction was collected over a year retrospectively and selection bias as the sample was self-selected and the two groups were recruited from different sources. The level of accuracy of BMI data in the BLW group is also questionable as anthropometric measurements were self-reported.

3.4.1.2 Diet

At 8 months, the foods consumed the most by all infants were breast milk and fruit, followed by vegetables, dairy products and cereals. Although in most studies baby cereal seems to be the most popular first food choice, the latest infant feeding survey found that mothers who introduced solids at 5-6 months were most likely to first offer fruit and vegetables to their infants compared to mothers who introduced solids earlier (McAndrew et al. 2012b). When comparing quantities of foods consumed with the national diet and nutrition survey for infants and young children there are similarities in intakes of vegetables (average intake in consumers 76g at 7-9 months versus 70.4g at 8 months in this study), fruit (mean 54g versus 95g) meat (16gr versus 13g) and fish (3 g versus 4.7 g). There were higher intakes of lentils and pulses in our group compared to infants from the infant diet and nutrition survey (mean intakes of 9.3gr versus 3 gr at 7-9 months)(Lennox et al. 2013). However there were methodological differences between the two studies in terms of dietary data collection which make the data not directly comparable. When compared with infants of the same age from the ALSPAC cohort, where the same methodology in dietary data collection was used, differences were also found in food consumption. Intake of cow's milk in the breast-fed babies' study was much lower than that of the ALSPAC cohort at 8 months (52gr among consumers versus 207 grams) with marked differences in whole milk consumption (36gr among consumers versus 226g). This could reflect the differences in infant feeding practices between the 90s and the present, although the official infant feeding recommendations did not recommend whole cow's milk as a main drink before the age of one year (Department of Health 1994). Differences in food intake are also likely to reflect socioeconomic differences between the two samples.

Infants in this study had lower mean energy, protein and carbohydrate intakes than the breast-fed subsample of the ALSPAC cohort (n=260) (Noble et al. 2001). Even though fat intakes were higher in this study (36g versus 31.5g), saturated fat intakes were similar for both groups which means that the difference in total fat intakes is made up of increased mean intakes of monounsaturated fatty acids (13g versus 11.3g), and less so polyunsaturated fats (5g versus 4.4g). Babies who followed baby-led weaning had higher intakes of polyunsaturated fatty acids than those who were spoon-fed (p=0.002). However this result is hard to interpret as there were no statistically significant differences between the consumption of fish and vegetable oils, main sources of PUFAS between the two groups of infants. A possible interpretation could be that the high PUFA intake was an accumulative effect of foods belonging to different food groups high in PUFAs such as hummus, tofu, soya products and nuts and hence the difference could not be detected at the food group level. Although dietary intake requirements for PUFAs have yet to be determined, they are very beneficial for growth and development including improved vision and behavioural and cognitive function (Koletzko et al. 2001; Gil et al. 2003). There are no set EARs and RNIs for carbohydrates, fats and fibre. In terms of expressing mean energy intakes as %EARs, boys just exceeded the expected EAR for their age groups with the distribution close to the EAR. Girls reached energy intakes just below EAR with the distribution close to the EAR set for their age and weight. The proportion of boys exceeding EARs for energy in this study were similar to those in the infant diet and nutrition survey (64% versus 65%), however fewer girls exceeded the EAR for energy in our study (33% versus 68%) (Lennox et al. 2013), The highest energy intake was 18% above the EAR and was observed in boys. Mean protein intakes were 1.5 times the set RNI with the distribution well above RNI, signifying that protein requirements were exceeded at this time point.

Mean vitamin intakes across the whole sample were generally within the recommended intakes at 8 months apart from niacin and vitamin D. Mean niacin intakes were only marginally lower than the recommended levels, however 60% of the sample had intakes below the RNI and 25% below the LRNI. The highest amounts of niacin derive from meat, fish and alternatives, followed by cereal and dairy products, potatoes and some vegetables. Consumption of foods from animal sources was relatively low at the 8 month time point which could explain the lower niacin intakes.

Intakes of all vitamins were higher in the national diet and nutrition survey for infants, however there were methodological differences and solids were introduced earlier than in this study (Lennox et al. 2013). When compared to the breast-fed subgroup of the ALSPAC cohort, intakes of all vitamins were higher in the latter group (Noble et al. 2001). Even though methodologically the two studies were comparable, 79.9% of the breast-fed group of the ALSPAC cohort were introduced to solids by 4 months of age (Emmett, North, & Noble, 2007), indicating that by 8 months their diet would be more established on solids foods than in the present study. Vitamin D intakes across all of the 3 infant populations were extremely low. In our sample all infants had intakes below the RNI of 7 μg/ day. In infants who followed baby-led weaning vitamin D intakes were significantly lower than the spoon-fed group. Vitamin D intakes were derived from food sources only and did not take into account the content of the vitamin in breast milk as it is uncertain (White & Allen 2013) nor the potential exposure to sunlight, which is the main source of vitamin D. This is common among this age group and especially in breast-fed infants who are one of the at risk groups for deficiency (Braegger et al. 2013). The Department of Health recommends vitamin D supplementation and vitamin drops containing vitamin D are included in the healthy start programme (Scientific Advisory Committee on Nutrition 2007b) and a recent study in the US also highlighted the need for supplementation especially in breast-fed infants (Perrine et al. 2010). However none of the infants in the study were taking supplements. As most infants are protected from exposure to sunlight in the first year of life, this could potentially have adverse health implications, not only for bone metabolism leading to rickets or other long-term consequences for bone density but also infectious diseases, allergy risk, cardiovascular disease and type 1 diabetes, although evidence is still scarce (Scientific Advisory Committee on Nutrition 2007b; Braegger et al. 2013; Elmadfa & Meyer 2012).

Infants who were introduced to solids after 6 months had significantly lower intakes of riboflavin, vitamin B6 and vitamin B12 compared to those introduced to solids before 6 months. In the case of riboflavin and B12 all infants who had intakes below the RNI were from those who were introduced to solids later. For vitamin B12 this was also true in infants with intakes below the LRNI. Riboflavin in baby-led weaned babies was also significantly lower than the spoon-fed group. These vitamins from the B complex are defined as Group I nutrients, that is to say their concentration in breast milk is dependent on maternal vitamin intakes or status (Allen 2012). Low levels of vitamins B6 and B12 have been reported in high income countries (Elmadfa & Meyer 2012; Allen 2005). It could be therefore that lower intakes of these nutrients in the first 6 months of life could have meant that once complementary feeding commenced at 6 months, these infants had already low levels. Main food sources of these B vitamins are animal products and fortified breakfast cereals. Coupled with the fact that complementary foods from animal sources were not consumed in large quantities and that the majority of the sample consumed organic breakfast cereals (and therefore not fortified with vitamins) could have contributed to the low overall intakes at 8 months.

In terms of minerals there were low intakes of calcium, phosphorus, iron and zinc at 8 months with all or almost all infants not meeting the recommended intakes. It is worth noting however that with the exception of iron which is discussed in a separate section, none of the infants had intakes of phosphorus and calcium below the LRNI, Zinc intakes in this study were lower than the ones reported in the infant diet survey (Lennox et al. 2013) but identical to intakes of breast-fed infants in the ALSPAC study (Noble et al. 2001). Dietary restriction of zinc has been linked with impairment in the immune system, weight gain and rate of growth (Krebs et al. 1994; Krebs 2000). Complementary feeding practices which are mostly plant based increase the risk of deficiency in older breastfeeding infants and therefore the early introduction of meat should be encouraged to avoid zinc insufficiencies (Krebs et al. 2006). In a study of 101 full-term infants aged 11.1 (±2) months, low zinc intakes were not different in infants introduced to solids before or after 6 months ($p=0.934$) which is also confirmed in our study, but they were significantly correlated to iron status (Park et al. 2012). The authors cite lack of meat intake as a possible cause which could also be the case for our study population or alternatively the fact that when the body is depleted of iron, it uses zinc for the production of haemoglobin (Park et al. 2012). Infants introduced to solids after 6 months had significantly lower intakes of selenium and infants who were following baby-led weaning had significantly lower intakes of iodine. These could be a reflection of later introduction of foods rich in these minerals such as meats and seafood.

Calcium and phosphorus intakes were lower than those reported in both ALSPAC and the infant diet survey. The majority of infants still relied on breast milk as a main source of milk which could have resulted in these low intakes, as breast milk is lower in calcium and phosphorus than formula milk or cows' milk and other dairy products. The combination of insufficient intakes of calcium, phosphorus and vitamin D could have implications in bone metabolism.

Mean intakes of sodium were similar between our study and the infant diet survey. Sodium intakes exceeded the RNI in 55% of the cases but according to the more realistic and achievable target of 400mg/day (equivalent to 1g salt) as recommended by SACN (Scientific Advisory Committee on Nutrition 2003) 30% of the sample (6 infants) had increased sodium intakes. This is considerably lower to the 70% of 8 month olds from the ALSPAC study who exceeded the SACN recommendations on sodium intakes (Cribb et al. 2012). Cribb et al reported the consumption of inappropriate foods in the highest quartile of sodium intake such as salty flavourings, gravy, baked beans and canned spaghetti. Our study is characterised by more homemade foods made from fresh ingredients and therefore that could explain the lower intakes as most of the salt in the diet is hidden in processed and ready-made meals. However, as with Cribb et al, there may also be a degree of underreporting and omitting, especially when reporting ingredients for homemade recipes.

By 18 months the establishment of a diet based on solely family foods was evident with the vast majority of infants consuming foods from all food groups, whilst infant specific foods such as formula and ready-made infant foods were very uncommon. Breastfeeding was still common for almost half of the sample. Food consumption at 18 months was therefore in line with current infant feeding guidelines for this age group.[1]

When comparing the food intakes with the infant diet and nutrition survey (Lennox et al. 2013) there were higher intakes in all food groups across the sample and also among consumers in our study. More specifically, the largest differences were observed across the sample in fruit (195.3 g versus 96g), pulses and lentils (39.1g versus 7g), fish (21.7g versus 12 g), eggs and egg dishes (31g versus 8 g) and meat (40.6g versus 29g). An interesting finding was that among consumers, breast milk intakes between the two groups were similar (290 g versus 287.8g) which could imply a similar subgroup in the infant and diet nutrition survey, however only 3% of the national sample were still breastfeeding between 12-18 months (assuming that by 18 months the proportion would be even smaller) as

[1] http://www.nhs.uk/start4life/Pages/babies-food-variety.aspx

opposed to 47% of infants in our sample. Beverages apart from water and milk were not consumed at 8 months (mean intake was 19g among 8 consumers), however at 18 months all infants were consuming beverages. There were 2 types of beverages consumed: unsweetened 100% pure fruit juice or 100% fruit smoothies and very small quantities of no added sugar squash, highly diluted with water. Quantities of these drinks were relatively low (mean intakes of 86g), which is consistent with similar studies such as the one by Lande et al who found significantly lower intakes of sweetened beverages and higher intakes of water in breast-fed infants compared to non-breast-fed infants (Lande et al. 2004).

Compared with infants of the same age from the ALSPAC study (Cowin & Emmett 2001) there were differences in all macronutrient intakes apart from protein. Infants in our study had higher intakes of energy, total carbohydrate and fibre, total fat and mono and polyunsaturated fatty acids. On the other hand intakes of saturated fats were lower than that of the ALSPAC cohort. The higher energy intakes could be therefore explained by higher intakes in beneficial macronutrients such as fibre, mono and polyunsaturated fatty acids which have proven benefits in gut and cognitive function respectively. The same pattern was observed for all macronutrients in the infant diet survey; demonstrating the differences between a predominantly breast-fed and a formula and/or mixed-fed population. Apart from one infant who was introduced to solids after 6 months and was spoon-fed, no other infants had energy intakes lower than the estimated average for their gender. As in 8 months, there were more than adequate protein intakes across the sample, consistent with the ALSPAC cohort, the infant diet survey as well as the rolling NDNS for children 1.5-3 years (Bates et al. 2012).

Vitamin intakes were satisfactory with mean intakes above RNI for most, with the exception of vitamin D where across the sample mean intakes only reached 31% of the RNI. This is higher than the latest rolling NDNS where mean intakes of vitamin D were 25% of the RNI (Bates et al. 2012) and below the infant diet survey in the breast-fed subgroup (37%). There was an improvement compared to vitamin D intakes at 8 months and considering the fact that none of the infants received dietary supplements it could reflect higher intakes of vitamin D containing foods such as fish, eggs and fortified cereals.

Babies who followed baby-led weaning had significantly lower Vitamin A intakes however, with 3 infants' intakes below the RNI, but none below the LRNI. Vitamin A has been characterised as a "problem nutrient" but this is mainly true for developing countries

(Elmadfa & Meyer 2012). Given the fact that overall nutrient status is more than adequate for all infants across the group the lower vitamin A intakes could reflect lower intakes of foods rich in vitamin A such as dairy products during the diet recording period.

Apart from iron which is discussed later, all other intakes of minerals were well above the recommended intakes. All but one infant exceeded the recommended intake for sodium, with mean intakes reaching 3.2g, well above the target of 2 g set by SACN for this age group (Scientific Advisory Committee on Nutrition 2003). This result is quite surprising, considering that in the infant diet survey mean salt intake for the 12-18 month groups was 2.3g (Lennox et al. 2013); this could however be reflecting the difference in age groups as food intakes between 12 and 18 month olds would be somewhat different. Also methodological differences between the two studies could also account for the deviation in intakes. The difference in number of days reported (3 days versus 4), although it would not have an effect on mean nutrient intakes it would affect % consumers. When compared with same age infants from the ALSPAC study where intakes were 3.5g for girls and 3.7g for boys (Cowin & Emmett 2001), this group had slightly lower salt intakes. Upon further examination, even though most foods were cooked from fresh and based on raw ingredients and none of the mothers added table salt, which is consistent with current UK practices (McAndrew et al. 2012a) there were intakes of some foods notoriously high in sodium such as canned soups, baked beans, marmite, cured meats and smoked fish which increased the overall salt content of the diet. Even though high salt intakes in adults have been linked with the development of hypertension (Scientific Advisory Committee on Nutrition 2003) its effect on health outcomes in childhood or any programming effect that tracks into adulthood are not known (Heird et al. 2006; Scientific Advisory Committee on Nutrition 2003). However it seems prudent to advise reduction of sodium intakes to those near the recommended targets. Iodine intakes were significantly lower in baby-led weaned infants compared to spoon-fed infants but this did not constitute cause for concern as intakes for both groups were well above the RNI.

3.4.1.3 Iron status

In the UK, the Scientific Advisory Committee on Nutrition (SACN) recommended further investigation in iron intakes and status in infants up to 18 months' age (SACN 2010). This is one of the very few studies which have looked at dietary and haematological data in the second half of infancy from breast-fed infants in the UK after the adoption of the recommendation for introduction of solids at 6 months.

Despite very low dietary iron intakes in this study, haemoglobin levels were well within the normal range at 8 months as described by the 50^{th} centile of Hb levels in the ALSPAC study (117g/l) (Emond et al. 1996) or in the recent diet and nutrition survey of infants and young children (115g/l) (Lennox et al. 2013). A study by Chantry and colleagues (Chantry et al. 2007) looked at the association between the duration of exclusive breastfeeding and iron deficiency anaemia. They found that in unadjusted analysis 10% of infants exclusively breast-fed for six months were diagnosed with anaemia as opposed to 2.3% of infants breast-fed for 4-5 months (p=0.007) and the association remained even after adjusting for birth weight and demographics. However, because anaemia was defined (among other things) as parental recall of history of anaemia it is possible that bias was introduced as these parents were more aware of the risk and possibly monitored their infants' iron status more regularly. We used the WHO criteria for low Hb levels due to a lack of any other internationally agreed cut-off point for HB levels in his age group, and by this criterion two infants were considered to have low iron status. However, this cut-off is widely considered to be too high. Using the alternative cut-offs based on population samples of both mixed-fed (Emond et al. 1996) and exclusively breast-fed infants (Domellof, Dewey, et al. 2002) none of the infants in our study would have been considered to have low Hb levels at 8 months. In contrast 7% of infants aged between 5-11 months had haemoglobin levels below 100g/L in the infants and young children's diet survey (Lennox et al. 2013).

There was no correlation between intakes of iron or other nutrients and haemoglobin in our sample. This is consistent with other literature and demonstrates the importance of other non-dietary factor in the utilisation of iron such as bioavailability and iron endowment at birth (Krebs et al. 2013). For example, a two minute delay in cord clamping significantly enhances iron status at birth and up to 6 months (MacMcDonald & Middleton, 2008), which means that potentially these infants would begin complementary feeding with an advantageous iron endowment.

The main finding was the extremely low dietary intakes of iron in this age group. Iron intake for most of the infants was well below the lower reference nutrient intake (LRNI), indicating that these intakes are only sufficient to meet the requirements of 2.5% of the general population (Committee on Medical Aspects of Food Policy (COMA) 1991). Low iron intakes in breast-fed infants of the same age were also observed among infants participating in the ALSPAC study in the 1990s (Hopkins et al. 2007), although intakes estimated by the same dietary method were somewhat higher than our findings (6.2mg/day in breast-fed and 9.0mg/day in formula-fed infants at 8 months). One possible explanation for this may be the popularity amongst our sample of organic infant foods which have become more widely used over the last two decades (The Soil Association 2012), while use of non-organic fortified infant foods was higher in ALSPAC. In the recent infants' and young children's survey (Lennox et al. 2013) mean iron intakes at age 7-9 months were 7.3mg/day (94% RNI); however the majority of iron in this group came from infant formula (48%) and commercial infant foods (20%) which are fortified with iron, whereas the infants in our sample consumed mostly family foods. This may also explain the difference in the proportion of infants below the LRNI between this survey (14%) and our data (70%). More recently, a study by Krebs (Krebs et al. 2013) looking at iron intakes and status in 3 groups of infants receiving 3 types of complementary foods (iron and zinc fortified cereal, iron fortified cereal and meat), found that iron intakes at 9 months ranged from 11.8 (1.3) mg/day in the iron and zinc fortified cereal to 7.5 (1.3) to iron fortified cereal and 3.3 (0.4) mg/day in the meat feeding group.

In the recent SACN report on iron and health, the issue of high incidence of iron intakes below the recommendations/LRNI was highlighted. The high proportions of certain population groups (namely toddlers and women of reproductive age) with dietary iron intakes below the LRNI coupled with low incidence of iron deficiency anaemia suggested that recommendations for iron intakes in these age groups may have been set too high (SACN 2010). Another possible explanation could be the potential increase in dietary iron absorption in response to low systemic iron (Domellof, Lonnerdal, et al. 2002) as seen in low serum ferritin levels; this effect is more prominent for non-haem iron (Lynch et al. 1989) and could explain why our sample did not show evidence of iron deficiency even though the majority of their dietary iron intake originated from non-haem sources.

In the study by Krebs (Krebs et al. 2013) 36% had mild anaemia (<115g/L) and dietary iron was not found to be correlated to serum ferritin within or among feeding groups (P=0.30). All groups had marginal iron status despite higher intakes in the fortified cereal groups, which lead the authors to conclude that there were differences in bioavailability of haem and non-haem iron.

In the ALSPAC study the incidence of anaemia at 8 months was 7.1% (ALSPAC cut-off 100g/l) or 22.7% (WHO cut-off 110g/l) (n=1079 infants). The incidence of anaemia was higher in the breast milk group (which consisted of breast milk with or without some cow's but no formula) than the formula and cow's milk group (Hopkins et al. 2007). In our sample the contribution of formula and breast milk to iron intakes was very small compared to other food sources and therefore the two samples are not comparable in that respect. In the ALSPAC breast-fed group, no associations between any nutrients and Hb were found even after controlling for confounders (gender, birth weight, age, weight, recent infections as assessed at the 8-month clinic, parity, maternal education, vegetarian status and use of iron supplements during pregnancy) (Hopkins et al. 2007), which is consistent with our data.

Despite the small sample size we were still able to demonstrate that iron intakes by these infants were significantly below the LRNI. All the infants were of normal birth weight, and it has been longstanding practice in Brighton to delay cord clamping, both of which are likely to mean that their infants would have had good iron stores at birth (Chaparro et al. 2006). It may be that an impact of such low iron intakes on haemoglobin levels would have been more apparent among infants with a lower iron endowment, e.g. as a result of low birth weight, early cord clamping (Dewey & Chaparro 2007) or if haemoglobin was measured at an older age after more sustained low iron intakes. Alternatively, it is possible that iron requirements after 6 months have been overestimated, the lack of sound evidence underlying recommendations on infant iron intakes has recently been highlighted (Scientific Advisory Committee on Nutrition 2010).

Haemoglobin levels at 18 months in our sample were higher in than those reported in the latest infants' diet survey (117g/L) with the breast-fed infants having a lower incidence of low haemoglobin 7.7% versus 15% below the cut-off point of 110g/L. (Lennox et al. 2013). The infants who had lower Hb levels at 8 months were found to have normal levels at 18 months. This was also observed in the ALSPAC study where infants with extreme low Hb at 8 months had normal Hb at 12 and 18 months (Sherriff et al. 2001). However our values of low haemoglobin were marginal as opposed to extremely low. Poor iron status in the first year has been associated with lower developmental scores at 6 years in Icelandic children (Gunnarsson, Thorsdottir, Palsson, & Gretarsson, 2007). Data from the ALSPAC study which followed infants at 8, 12, 18 months measuring haemoglobin levels and developmental outcomes found that low haemoglobin at 8 months (below 95g/l) was significantly associated with negative developmental outcomes at 18 months, whereas this was not observed from low Hb levels at 12 and 18 months, suggesting a vulnerable period at around 8 months of age which affects future development (Sherriff et al. 2001). Dietary iron intakes were also higher in our sample [6.7 mg/day (97.1%RNI) versus 6.4 mg/day (93% RNI)], with no children with intakes below the LRNI as opposed to 13% of infants below the LRNI in the national infant diet and nutrition survey. There were differences in the contribution of food groups to iron intakes with our sample obtaining most of it from family foods whereas in the diet survey 17% of iron came from formula and 9% from commercial infant foods. A study of 6-9 month old healthy non anaemic Swedish infants looking at the mode of iron intake (medicinal drops versus dietary iron) showed that iron from fortified foods is incorporated into haemoglobin rather than deposited into iron stores unlike medicinal iron which is converted to ferritin. This is potentially due to the role of hepsidin. A large one off peak of iron mobilises hepcidin production which increases iron storage whereas the low level ingestion of iron through iron rich complementary foods throughout the day uses a different mechanism of iron utilisation by incorporating it into haemoglobin production (Domellof et al. 2008). This could explain the higher haemoglobin levels at 8 months but it may also explain a longer lasting impact on haemoglobin at 18 months after dietary iron intakes have increased markedly from 8 months.

3.4.1.4 Eating behaviours

All mothers followed the current infant feeding guidelines and continued breastfeeding at 8 months of age. This is consistent with the sample demographic and the wider literature which suggests that older, more educated women are likely to breastfeed for longer (McAndrew et al. 2012b). The timing of the introduction of solids was also in keeping with the guidelines (British Dietetic Association 2013a) with all infants introduced to solids after 4 months.

A third of the sample used the baby-led weaning approach, bypassing puree textures and starting straight with finger foods. All of the infants that followed this approach were introduced to solids either on or after 6 months. Our findings are in line with a study which looked at characteristics of infants who followed the baby-led weaning approach and found that infants were introduced to solids later and participated more in family meals. They also found that mothers breast-fed longer, even after adjustment for socioeconomic status (Brown & Lee 2011a).

By 8 months, all mothers but one had introduced foods with lumpy texture in the infants' diet. Late introduction of lumpy solids at 10 months has been shown to increase feeding problems (Northstone et al. 2001) which can potentially persist into childhood (Coulthard et al. 2009) however lumps in our sample were introduced between 6-8 months. Finger foods were also introduced around 7-8 months by the majority of the sample, in accordance with current recommendations.

Mothers reported recognising their infants' signs of physical and behavioural readiness before they proceeded with introducing solids. These responsive cues, which indicated a positive mother-infant interaction, mirror results from another study by Brown and Lee in which mothers who were interviewed about their experience in baby-led leaning said the introduction of solids was prompted by their infant's perceived developmental readiness, indicating a responsive approach to the infant's behaviour (Brown & Lee 2013).

Conflicting health professional advice was evident, as some mothers reported that were told that by 6 months the infant should have a full eating routine; therefore they were advised to introduce solids 2 weeks earlier than 6 months. The Department of Health advice states that introduction of solids should be gradual and although timescales are not provided it does not assume a full eating schedule at 6 months. This could potentially be problematic because parents could get unnecessarily distressed if they do not manage to establish an eating routine by 6 months as per the health professional's advice. In a study exploring maternal views on what influences their decision on when to introduce solids, advice from health visitors was shown to be inconsistent compared with current WHO guidelines and later introduction of solids was associated with rating health visitor advice as poor (Arden 2010). This was also evident in another study by Redsell where parents reported that health visitors often supported the idea of supplementation of breast milk with formula, which for some participants who wanted to persist with breastfeeding was seen as inappropriate and unwanted advice (Redsell et al. 2010). This is also reflected in the latest infant feeding survey; even though the health visitor is still the main source of information about when to start giving solids (65%) there is a big decrease compared to the 2005 survey (87%). Books/leaflets/magazines, friends/family and the internet are playing a much bigger part in the solids introduction decision process than they did 5 years earlier (McAndrew et al. 2012b).

More mothers in our study said their infant would eat almost anything compared with data from the latest infant feeding survey (80% versus 72%) (McAndrew et al. 2012b). Infant flavour exposures begin before complementary feeding through maternal diet during pregnancy (amniotic fluid) and breastfeeding (Cooke & Fildes 2011). Unlike formula milk, whose composition is consistent, breast milk varies depending on maternal diet, resulting in exposure to different flavours during breastfeeding. Breastfeeding may therefore constitute a positive experience in accepting new flavours and learning to eat a variety of foods (Birch et al. 2007). It is reasonable to expect that breast-fed infants will have a greater exposure to different flavours. A study by Shim and colleagues (Shim et al. 2011) who looked at infant feeding guidelines and prevalence of fussy eating behaviours found that exclusive breastfeeding for six months was protective against food rejection (fruit, veg, meat, fish) (OR 0.19, CI 0.06, 0.69), preferences for specific food preparation method (OR 0.22, CI 0.06, 0.81) and food neophobia (OR 0.25, CI 0.07, 0.89). Also introduction of solids before 6 months increased the odds of food neophobia (OR 2.45, CI 1.01, 5.93) and having a limited variety of food (OR 2.46, CI 1.06, 5.73) after adjusting for mother's age, race, marital status, employment status, and education level, household income level, child's age, sex, and ethnicity. The authors hypothesized that the theory of "learnt safety" could be a possible mechanism for this: infants who are introduced to solids early are more likely to experience feeding problems due to gastrointestinal immaturity. If these problems occur often, then the infant is more likely to associate foods with gastrointestinal discomfort and be more reluctant to try new foods (neophobia), therefore limiting the variety of foods eaten (Shim et al. 2011).

When asked about specific feeding problems, insufficient food intake was the one that worried mothers "a bit". Food refusal, fussiness and establishing routine, although reported did not worry them. A very small proportion of mothers worried greatly about overeating. These figures demonstrate a level of certain confidence in how mothers perceived infant feeding difficulties at 8 months. Maternal pressure to eat can have adverse effects in the infant's food intake and increase levels of fussy eating. Parental influence is bidirectional: the parent affects the eating environment of the child and at the same time the child's behaviour is shaping parental response (Ventura & Birch 2008). Moreover, mothers who followed baby-led weaning used a significantly less restrictive and pressurised feeding approach and were less concerned over their infants' weight compared to those using a traditional weaning method (Brown & Lee 2011b). In terms of difficulties in feeding solids to their infants, more mothers reported feeding difficulties than those reported in the 2010 infant feeding survey (20% versus 11%). More specifically in the infant feeding survey 17% of infants who were introduced to solids between 5-6 months and 6 months reported difficulty as opposed to 10% of infants who were introduced to solids between 4-5 months. According to the latest infant feeding survey (McAndrew et al. 2012b) almost half of the mothers (45%) reported actively avoiding giving their infants at least one ingredient as opposed to 65% in our sample. Food avoidance at 8 months yielded interesting results with over a third of mothers in our study actively avoiding red meat. This is against UK guidelines which suggest introducing iron-rich foods as soon as solids are introduced in the appropriate texture for the age of the infant (British Dietetic Association 2013b). However this message seems to have not been well communicated even to this highly educated group of mothers. Results from the 2010 infant feeding survey with regards to meat avoidance were lower compared to ours and showed that 6% avoided meat in general (offal/chicken/ham), 1% beef and 5% other red meat (pork, lamb etc.). The potential impact of this on the infants' iron status was discussed in the previous section. In the baby-led weaning group, significantly more mothers were not avoiding giving nuts to their infants compared to the mothers who did not follow a baby-led weaning approach demonstrating compliance with the most up to date guidelines on the subject. Although more research is still needed to determine the effect of the age of introduction of allergenic foods in the development of atopic disease (Grimshaw et al. 2009) current consensus is against the early (before 4 months) or late (after 7 months) introduction of such foods as eggs, fish and nuts (Agostoni et al. 2008; Shreffler & Radano 2011; Anderson et al. 2009). In the UK, mothers are advised to exclusively breastfeed for 6 months and -apart from those who have a suspected or proven food allergy or other allergic disease- to start introducing allergenic foods as soon as weaning has become established with traditional foods. Allergenic foods are advised to be introduced in small amounts , one food category at a time (for example wheat products) and spaced out over 2-3 day intervals (British Dietetic Association 2010).

Significantly more mothers in the late solids introduction group said that their infant ate differently at 8 months but the majority described the difference as following baby-led weaning. Baby-led weaning was becoming very popular at the time of the study. A few studies have looked at parental perceptions of baby-led weaning which include mostly positive reports of being a more natural and healthy way of introducing solids and that infants take part in mealtimes and eat family foods rather than foods especially prepared for them (Cameron et al. 2012; Brown & Lee 2013).

A large proportion of mothers were still continuing to breastfeed at 18 months with the earliest breastfeeding cessation being at 9 months of age. This is very encouraging and in line with the World Health Organisations' guidance to continue breastfeeding after the introduction of solids at 6 months for 2 years or beyond (World Health Organisation 2002). The latest information from infants in the UK shows that only 8% of mothers report breastfeeding their infants aged between 12-18 months (Lennox et al. 2013). However Brighton and Hove has breastfeeding initiation rates that are above the national average (86% versus 76% in 2008/2009) and have lower drop off rates between initiation and 6-8 weeks (13.4%, ranked 4th nationally in quarter 3 2009/2010 with the best rates in that quarter at Westminster, 8.7%) (Williams & Pickin 2010) so our data is consistent with information from the local area.

One mother worried greatly about their infant not eating sufficient food at 18 months however from our anthropometric data, we did not have any evidence of underweight (weight-for-age z- score less than 2) in any of the infants. In a study of assessing maternal feeding practices with feeding behaviours in 12-36 month old infants although 63% felt that their child was easy to feed, 20% perceived their infants as a fussy eater. 23% worried whether the child was eating sufficient food, whether the child had a small (17%) or large appetite (6%). Food refusal was common in 20% of the sample and occurred sometimes in 42% of the sample (Chan et al. 2011).

Encouragingly, fewer mothers were avoiding foods at 18 months with all infants having gluten and fish in their diet and only 3 mothers actively avoiding red meat. These positive signs are in line with other studies that confirm that infants who were introduced to solids later incorporate family foods more easily, have a wider variety of foods and are less fussy; mothers whose infants followed baby-led weaning reported sharing mealtimes and adjusting them around their infants hunger patterns. The infants were offered the same food as family food, accordingly adjusted (low salt, low additives) but the addition of spices and herbs was common. The mothers in this study believed that offering a wide range of foods and giving the infants the choice to feed themselves could promote a healthier approach to food and result in their infants' being less fussy and eating a wider variety of foods in the future (Brown & Lee 2013).

3.5 Limitations

The main limitation of this study was the small sample size. It comprised a small self-selected group of mothers, nearly all of whom were educated to at least degree level; however, data from the last infant feeding survey suggest that this is typical of the very small percentage of mothers who breastfeed for at least 6 months (McAndrew et al. 2012a). It was therefore not possible to conduct adjusted analysis in order to account for potential confounders and this is a major disadvantage of the study. However, the sample was fairly uniform in terms of parental socioeconomic status and type of milk feeding which constitute two of the major confounding factors and therefore there is some confidence that the results may be reflecting a true association, and therefore providing some initial indications that future studies could build upon.

The sample of this study consisted of a group of infants with very high breastfeeding intakes who were introduced to solids much later than the national average and consumed mainly fresh, home prepared foods. Therefore the results are not representative of the UK infant population. However, the feeding practices of these infants are highly consistent with current UK guidelines and therefore their intakes can be interpreted as a "target" that all infants should be aiming for.

Maternal reports of perceived fussy eating and other feeding behaviours were used. A recent review found a lack of good quality, reliable validated questionnaires assessing infant feeding behaviours with no questionnaires looking at food avoidance and food approach (including fussiness, rejection, food enjoyment) between 12-24 months. There were also no questionnaires assessing parental control in feeding practices in infants younger than 2 years (de Lauzon-Guillain et al. 2012). However, Jacobi and colleagues assessed parental reports of fussy eating using objective laboratory methodology and demonstrated that parental perceptions of feeding difficulties and pickiness corresponded to clinical measures of restricted dietary variety and limited diet patterns (Jacobi et al. 2003).

Another limitation as with every survey which involves assessment of dietary intakes was misreporting. However this group of well-educated, heath aware mothers were extremely motivated and interested in the study and were given detailed information on food portion sizes therefore were less likely to misreport intakes. We did not find evidence of unusually low or high intakes of nutrients which further supports the accuracy of dietary recording. Additionally, using energy adjusted intakes is also an effective method for accounting for misreporting when examining several nutrients (Poslusna et al. 2009).

The "gold standard" of dietary assessment is considered to be a seven day weighed intake (Bingham et al. 1994). It was felt that seven days of recording would place an unacceptable burden on the mothers participating in this study. The time between 6 and 8 months is a transition period from milk to solids and there is relatively small day to day variability (Stuff et al. 1986), meaning that reliable estimates of nutrient intake can be obtained with fewer days of dietary recording. Food records compare well with the results of weighed food intakes (Bingham et al. 1994), and results obtained by this method in earlier studies of infants have compared well to the results of national surveys, and had plausible relationships with biological outcomes including ferritin and blood lipids (Noble et al. 2001; Cowin et al. 2001; Hopkins et al. 2007; Rogers & Emmett 2001). Assessment of breast milk intake was based on maternal ability to record duration of breast feeds, as well as a crude method of calculating the total amount consumed. However, as breast milk only accounts for a very small proportion of the infants' iron intakes, any errors in estimates of breast milk intake would have only a minor impact on estimates of total dietary iron.

3.6 Chapter Summary

The small sample size of this study meant that there was no opportunity to conduct adjusted analyses and to generalise the results. Even though some significant differences were detected, it is not possible to draw firm conclusions on the association between the age of solids introduction and mode of complementary feeding with growth, diet, iron status and eating behaviours at 8 and 18 months. However, assessing health outcomes in a population that conforms to the current infant feeding recommendations makes this study novel and timely. It provided some interesting exploratory indications on health outcomes in this infant population that can form the basis for future research as well as useful insights of the challenges of conducting a study of this type which can help in the design of adequately powered studies in the future.

Chapter 4

The Haemospect® Validation Study

4 The Haemospect® Validation Study

4.1 Chapter Overview

This chapter will describe the use of a novel non-invasive device, Haemospect®, which measures haemoglobin levels using white light spectroscopy in a subgroup of the Brighton's Breast-fed Babies Study. Haemoglobin measurements obtained from the device will be compared with measurements from heel pricks as described in the previous chapter.

Aim, objective and research hypothesis

Aim 3: To test the accuracy of a white light spectroscopy device (Haemospect®) in measuring haemoglobin levels compared to a capillary venous blood sample.

Objective

To obtain Hb measurements by Haemospect® in principally breast-fed 18 month old infants from The Brighton's Breast-fed Babies study and to compare them to the Hb measurements obtained concurrently in the same infants using a heel-prick.

Hypothesis to be tested

There are no differences in haemoglobin measurements between the capillary venous blood sampling method and the Haemospect® method.

4.2 Methods

4.2.1 Study design

The study design was a prospective cross-sectional study ran as part of the Brighton's Breast-fed Babies Study. The methodology of the Brighton's Breast-fed Babies study was described in the previous chapter. In summary, healthy full-term infants whose mothers intended to breastfeed them for at least six months (at least 80% of milk intake deriving from breast milk) were recruited from Brighton and Hove. Anthropometric, dietary and haematological data were collected at 8 and 18 months.

4.2.2 Settings

The study was conducted during the last home visit at the participants' homes, as described in Chapter 3.

4.2.3 Participants

Inclusion and exclusion criteria have been described in the previous chapter. For the purposes of this study, toddlers aged 18 months took part.

4.2.4 Data collection

Haemospect® is a portable device manufactured by MBR systems (Germany). It consists of the hand-held analyzer and a button sensor (Figure 4-1). It can be operated on both battery power and mains electricity.

Figure 4-1 The Haemospect® device

The device was automatically calibrated before every use using the relevant setting. Once the device was ready to use, the forearm of the infant was disinfected using an alcohol swab and the probe was placed on the forearm. Gentle pressure was applied on the probe by the researcher to ensure that there was complete contact with the skin at all times. After 60 seconds the reading was obtained and recorded. The second reading was obtained using the method described above 1-2 minutes after the first measurement. The time lapse between the non-invasive method and the heel prick was 5-7 minutes.

Haemoglobin is not evenly distributed in capillaries and tissues because of changes in the distribution of capillary perfusion. When white light is projected through the skin, some of it is absorbed by the tissues and some is reflected back. The device captured the reflected light and a spectrometer separated it into its different wavelengths and analysed it. Then, using an algorithm, haemoglobin was calculated.

4.2.5 Variables

Two Haemospect® measurements were obtained for each infant. Haemoglobin measurements were obtained by a capillary venous sample taken from the infant's heel and analysed using a haemoglobinometer (Haemocue 201+). A detailed account of the blood sampling methodology can be found in Chapter 3.

4.2.6 Sample size calculation

Previous studies have calculated a sample size based on the design planning of comparison of laboratory methods (Rabe et al. 2010). It was estimated that 200 comparison pairs would be required in order to detect a difference of 0.8 at a 5% significant level (Rabe et al 2010). However due to lack of resources and time, this was not possible to achieve, therefore our sample size was 10.

4.2.7 Statistical analysis

For comparison with the heel prick measurements (set as the standard method), mean, standard deviation, median, inter-quartile range, and maximum and minimum values were calculated from each pair of Haemospect® measurements taken. The Bland Altman method was used to compare the two methods. More specifically for each infant, the Hb difference between the two methods was calculated. Then for each set of measurements the average was also calculated. These two variables were subsequently plotted against each other in a scatter plot (Figure 4.1). This plot therefore represents the relationship between the measurement error and the true value (which should lie somewhere in between the two measurements). The standard deviation of the difference between measurements was also calculated in order to provide the distribution range of 95% of the values (mean +/- SD)(Bland & Altman 1999). The two methods were also regressed against each other and their relationship is represented in Figure 4.2.

4.2.8 Ethical Approval

The study was awarded a favourable ethics opinion by the Kent Research Ethics Committee and NHS Brighton and Hove and South Downs PCT Research and Development Departments (Appendix 1).

4.3 Results

4.3.1 Participants

Participants who consented to the blood sample at 18 months (n=14) were also invited to participate to the validation study. Written informed consent was obtained for both procedures separately so that participants had the option to opt out of either one. Eventually 10 pairs of capillary and non-invasive measurements were taken. (Non-availability of the Haemospect® device for n=3 and child feeling unwell n=1 for the data not collected). A mean value of 2 Haemospect® measurements was calculated for each infant. In one case that was not possible because the infant was not compliant during the second measurement. The first measurement was therefore carried forward in that case.

4.3.2 Descriptive data

Both capillary and Haemospect® readings were normally distributed. Mean age of infants when the measurements were taken was 17.5 (SD: 0.5) months. Four infants (40%) had parents of non-white origin (African, Indian or mixed race) which meant that the light reflected from the device would be affected by the melanin content of the skin. Table 4-1 shows means and distributions of the two measurement methods. The Haemospect® method had consistently higher readings than the heel prick method, with values ranging from 14.6-15.2 g/dL.

Table 4-1 Comparison of means and distributions of hemoglobin levels from alternative measurement methods

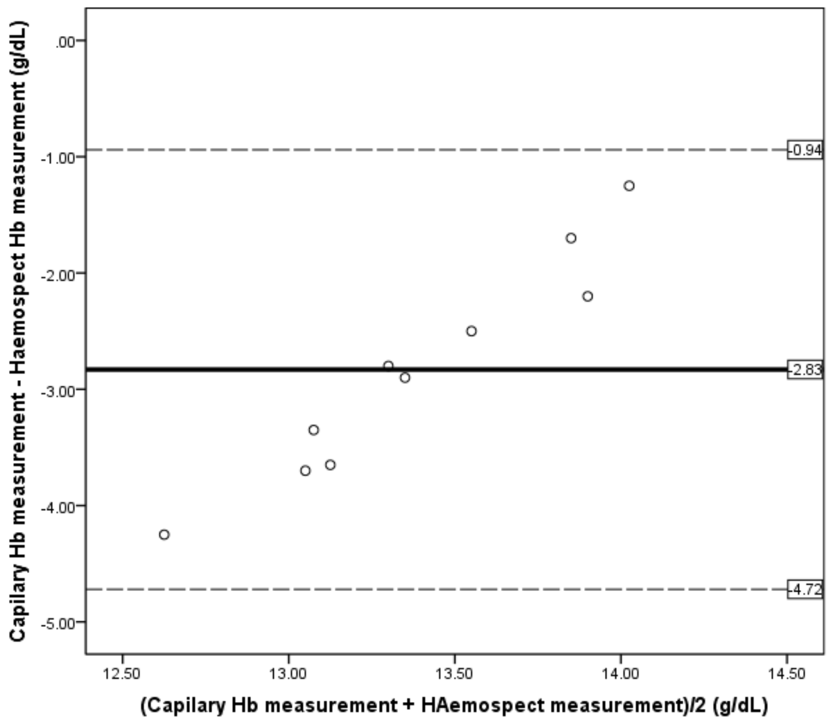
Hb measurement method	Haemoglobin concentrations (g/dL)						
	Min	**Mean**	**SD**	**25%**	**50%**	**75%**	**Max**
Capillary measurement	10.50	11.97	0.91	11.28	11.90	12.85	13.40
Haemospect® measurement 1	14.60	14.79	0.11	14.70	14.80	14.83	15.00
Haemospect® measurement 2	14.70	14.81	0.16	14.70	14.75	14.90	15.20
Mean Haemospect® measurement	14.65	14.80	0.12	14.70	14.78	14.91	15.00

n=10 for all measurements

4.3.3 Main Results

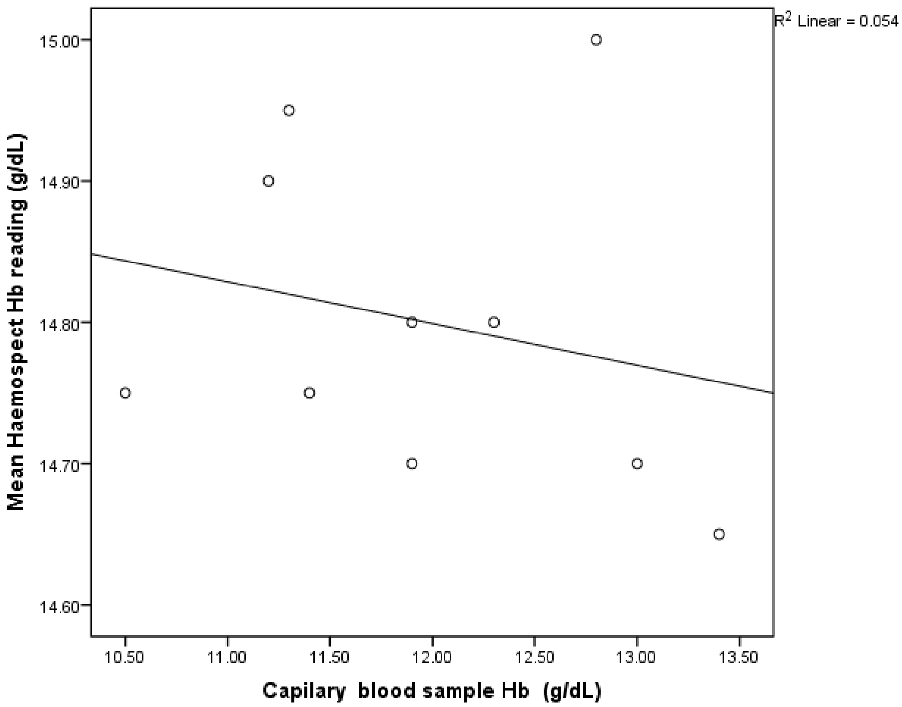
Looking at the Bland Altman plot in Figure 4-1, although the agreement limits are within an error of 5% of the SD (0.9 g/dl) as indicated by the dotted lines on the graph, the mean difference between the two methods is displaced away from 0 (Value of -2.83, indicated by the dark line on the graph), further confirming the overestimation of Hb by the Haemospect®. The regression line shows that there is no correlation between the two measurements (*Haemoscpect Hb = -0.03 Capillary Hb +15.15*) p=0.517 with a very low R^2 value (0.052).

Figure 4-2 Bland-Altman comparison for capillary and spectroscopic haemoglobin values (both in g/dL).



The mean difference between the two methods is denoted with the dark line (-2.83), and the ±2 standard deviation (SD) limits (-0.94, -4.72) where SD:=0.9, are denoted with the dotted lines.

Figure 4-3 Scatterplot of the values for capillary blood haemoglobin (Hb) concentration (g/dL) versus the corresponding values from Haemospect®



4.4 Discussion

The aim of this study was to compare a non-invasive method for assessing Hb levels in 18 month old toddlers to a standard capillary blood sampling method. Looking at the descriptive results it was evident that Haemospect® consistently overestimated Hb levels in all infants, compared to the heel prick results. This was also confirmed in the Bland Altman plot where the differences between the two measurements were increasing as the mean Hb values got larger. Results from the regression analysis showed that there is not a strong correlation between the two methods. Therefore the Haemospect® was deemed unsuitable to detect low haemoglobin levels in this age group.

The novelty of this study was that this is the first one to assess a non-invasive method of haemoglobin measurement in the community in a group of 18 month old toddlers. Other attempts for measuring haemoglobin using noninvasive techniques have been made in adults, for example using conventional near infrared spectroscopy with low levels of sensitivity (Rendell et al. 2003) and more recently pulse oxymetry (Barker & Badal 2008) with very promising results in the continuous measurement of haemoglobin in hospital settings (Bamberg 2008). The Haemospect® has been used in neonatal units and compared to both venous and capillary blood samples with very high correlation coefficients (R^2=0.96 for capillary/spectroscopic and 0.99 for venous/spectroscopic pairs respectively) (Rabe et al. 2010). Possible reasons for the lack of accuracy in our sample could include the fact that skin in toddlers is much thicker than neonates and therefore it could be that the light was not reflected back or absorbed properly during the measurement. Also skin colour can affect the results as the light absorption is affected by melanin in the skin and as 40% of our sample had one parent of non-white origin this could have also played a part in the inaccuracy of the results.

The device was handheld and easy to carry and handle during home visits so if proven accurate it would be suitable for use in community settings. The device was very easy to use and training required was only 30 minutes. Its use could potentially eliminate blood loss, pain, skin damage, risk of infection and distress and would potentially increase the number of parents willing to screen infants for anaemia, leading to early detection of anaemia.

4.5 Limitations

The main limitation of the study was the small sample size which was due to the already limited number of infants for the Breast-fed Babies Study as well as the delay in obtaining the device from the manufacturers. The implications of the small sample size have been discussed in Chapter 3 and Chapter 6. Therefore larger scale studies are needed to improve the strength of the results.

4.6 Chapter Summary

In conclusion, the Haemospect® device failed to detect low haemoglobin levels in this group of 18 month old toddlers. Adjustments to the device and further testing in a larger sample of the same age in a community setting are recommended. However, even though the sample size of the study was small, the complete lack of correlation between the device readings and the heel prick measurements indicate that the device may not be suitable for use in this age group.

Chapter 5

The GO-CHILD Study

5 The GO-CHILD Study

5.1 Chapter Overview

This chapter will examine the association between the age of solids introduction with some of the same eating behaviours assessed in the Brighton's Breast-fed Babies Study in a larger sample of infants as well as other aspects of the complementary diet, namely food preferences and dietary variety. The results will then be discussed and placed within the context of current literature.

Aim, Objectives and research hypothesis

Aim 4: To explore eating behaviours, fruit, vegetable and meat preferences and dietary variety at 9 months between mixed-fed infants from Sussex who were introduced to solids before and those introduced to solids after 6 months.

Objectives:

- To identify the presence of parent-reported feeding difficulties and other eating behaviours across the whole sample and compare differences between the early and late complementary feeding groups.
- To assess the level of acceptance of fruit, vegetables and meat across the whole sample and compare differences between the early and late complementary feeding groups.
- To examine the variety of different fruits, vegetables as well as of the overall diet across the whole sample and compare differences between the early and late complementary feeding groups.
- To assess if the age of solids introduction is an important predictor of eating behaviours, fruit, vegetable and meat preferences and dietary variety at 9 months after adjusting for other confounding factors.

Hypothesis to be tested

There are no differences between eating behaviours, fruit, vegetable and meat preferences and dietary variety at 9 months between mixed-fed infants from Sussex who were introduced to solids before and those introduced to solids after 6 months.

5.2 Methods

5.2.1 Study Design

GO-CHILD is a prospective multicentre cohort study which aimed to assess the genetic and environmental factors (including diet) that affect childhood diseases such as asthma and allergies. A database containing information about genotypes, environment, and disease and health outcomes was set up antenatally and was completed with blood and saliva samples from infants for genotyping at birth. The aim of the study was to collect detailed information on asthma and other allergy outcomes in the first two years of life and explore the gene-environment interactions relevant to asthma, allergy and other childhood diseases.

5.2.2 Settings

Recruitment began in 2009 and the study was conducted in several sites in Sussex, Surrey, Fife, Tayside and Warwickshire.

5.2.3 Participants

At all the sites, an information leaflet about the study was given to all expectant mothers at their 12 week scan appointment which was part of the routine care for all pregnant women or at any other antenatal /scan clinic visit from 12 weeks gestation onwards. Leaflets were also posted to all expectant mothers in the study areas.

5.2.4 Measures

Women were asked to join the study at the antenatal clinic/ scan visits or home visit around 12 weeks onwards. Those who agreed to join the study, were sent a letter at 30-35 weeks reminding them that they had agreed to join the study and offering them the opportunity to withdraw. For mothers who consented, historical information was collected by administering a simple questionnaire to the mother, her partner and the biological father if available. Additional information was also obtained from the mother's medical records if required. The mother was also given a self-completion food frequency questionnaire describing her diet during this pregnancy.

A cord blood sample was collected from infants of consenting mothers delivered at all sites by the attending midwife or other staff, or by one of the researchers. The collection of cord blood did not cause any pain or discomfort to either mother or infant and took about 1 minute. If a cord blood sample was not obtained, the researcher approached the mother on the postnatal ward (or neonatal unit if the infant was admitted), in order to collect a saliva sample from the newborn. If she consented, a saliva sample was taken from the infant. Cord blood, together with the umbilical cord and placenta is normally discarded after birth. The extraction of DNA from cord blood thus represents a suitable strategy to establish a genetic database that can be subsequently merged with phenotypic data that is captured at various points from the antenatal period to childhood in order to gain an understanding of the gene-environment interactions that may control the onset and severity of allergy-related disease. For infants born at the Sussex County Hospital, lung function, oxygen saturation and skin micro-vascular function were measured in the infant non-invasively during the first 3 days after birth, while the child was in the post-natal ward. The parents had the option to opt out of this part of the study, if they wished to. The aim of this part of the study was to understand the development status of the infants' lungs at birth and to check the status of the microvasculature of the skin. Both tests were non-invasive and did not carry any risks for the infant. The infants were followed up at the ages of 3, 6 and 9 months, 1 year and 2 years. For infants born at the Sussex County Hospital, a stool sample at ages 3, 6, 9, 12 and 24 months was also collected, to investigate the presence of *Clostridium Difficile (C.Diff.)* in the infant gut influencing the atopic outcome, and whether the carriage/clearance of *C. Diff* in infants was related to genetic polymorphisms in the innate immune system. For these infants, the measurement of lung function, oxygen saturation and skin micro-vascular function was repeated at age 1 and 2 years. At 3 months parents were sent a questionnaire focussing on infant feeding patterns and growth up to the age of three months. At 6 months the questionnaire sent focused on infections experienced, GP and hospital visits and symptoms of allergy and wheeze. At 9 months the questionnaire assessed breastfeeding, formula feeding and patterns of solids food introduction. At the ages of 1 year and 2 years a validated questionnaire developed in Leicester and Nottingham to assess the incidence of wheeze, allergy and eczema in young children was administered. The questionnaires were posted to the mothers with prepaid envelopes for return. Telephone and postal reminders were provided to parents who failed to return the questionnaires within two weeks.

The dietary information collected at 9 months by the Go-CHILD team involved infants of a similar age with those of the Brighton's Breast-fed Babies Study, from the same geographical area and included information on age of solids introduction, eating behaviours and food frequency. Since the small sample size of the BBBS did not enable us to draw safe conclusions for our research questions, it was thought that an adequately powered study such as the Go-CHILD would provide a useful addition to the evidence base. The involvement in the Go-CHILD study for the purposes of the thesis therefore was to approach the GO-CHILD team and liaise with them in order to obtain the 9 month questionnaires, to build a database and enter the data that were already collected from other members of the research team, to form the research questions and devise an analysis plan based on the best way that the available data could answer the original research questions as well as explore other outcomes such as variety and food preferences. The data was then analysed and the findings are reported in the following sections.

5.2.5 Study size

The following power calculation was conducted for the main outcome of the Go-CHILD study. Using conventional criteria for confidence level (95%) and power (80%) and estimates of the proportion of children with a positive skin prick test as 20% in children without the filaggrin mutation (the most significant genetic risk factor for the development of atopic dermatitis) and 40% in those with the mutation the total sample size needed was 166 (83 babies with and 83 babies without the filaggrin mutation). Accounting for losses to follow, a total sample of 200 babies was estimated. However for the purposes of the thesis' outcomes a post hoc power calculation will be performed.

5.2.6 The 9 month diet questionnaire

The 9 month diet questionnaire included information about infant feeding practices, age of solids introduction, introduction and consumption of specific food items and eating behaviours such as feeding problems and perceived fussy eating. The questionnaire can be found in Appendix 2. The extraction and processing of the questionnaire variables are described in the following sections.

5.2.7 Assessment of food preferences

Food preferences are defined as foods that infants are perceived to like or dislike. De Lauzon-Guillain and colleagues (2012) reviewed tools developed to measure eating behaviour and food intake or preferences of preschool children. However they found that no tools were developed for children under 18 months of age (de Lauzon-Guillain et al. 2012). Parental reporting of infants' food preferences (likes and dislikes) has been used in other studies (Skinner et al. 2002).Parents were therefore asked how much their infants liked three types of foods; vegetables, fruit and meat. Due to their different sensory properties and therefore different levels of acceptance, fruit and vegetables were considered separately as also noted by Glasson and colleagues (Glasson et al. 2011). Infants' preferences were measured on a 4 point Likert style scale with the following options: "Likes this very much" "Quite likes this", "Does not really like this" and "Will not eat this". There was also a fifth option "Has not tried this" for foods not yet introduced to the infants. For the purposes of the analysis, the "has not tried this" option was excluded and the 4 remaining categories were then merged into 2: "Likes food" constructed from options 1 and 2 and "Does not like food" constructed from options 3 and 4.

5.2.8 Dietary variety

There is a lack of consensus when defining dietary variety or diversity (two terms that are used interchangeably in the literature). Dietary variety can be defined as the number of foods or food groups consumed over a specific time period (Ruel 2003). In this study food variety was defined as the number of new foods introduced to the infant from the beginning of solids introduction until the age of 9 months. This definition has been used before by Lange and colleagues (Lange et al. 2013). Variety within food groups (regardless of the quantities consumed) was calculated as follows. Each food item from the questionnaire was categorised in one of the following core food groups: starchy foods, fruit and vegetables, dairy foods, meat and protein foods (Table 5-1). Fruit and vegetables were examined both separately and as one category. The food groups and the number of food types within each group are shown in Table 5-1 along with a complete list of food types in each group. Because of the nature of the questionnaire some individual food items were already grouped together and therefore could not be distinguished from one another. For example, "Oranges/satsumas/kiwis/strawberries" were considered 1 food type for the purpose of this analysis. A cut-off point to signify low versus high dietary variety was set. According to Ruel, cut off points should be defined using the dietary patterns of the population examined and when looking at associations between variety and health outcomes, these should be based on the internal distribution of diversity within the study sample (Ruel 2003). Looking at the distributions of vegetable, fruit and overall dietary variety none were normally distributed; in fact all of them were highly positively skewed.

Log transformation and square root transformation was performed, however the distributions remained non normal. Previous studies with similar issues have used the 75th centile of the distributions to dichotomise the scores into low and high variety (Howard et al. 2012). However in our case, the 75th centile contained the same values as the 50th centiles for vegetables and therefore it was decided to use the 25th centile as the cut-off point. For consistency, the 25th centile was used for the other two variables as well. The 25th centile corresponded to consuming 5 of 7 vegetable types (16.1% of population had low variety versus 83.9% with high variety), 3 of 5 fruit types (27% of population had low variety versus 73% had high variety), and 26/51 foods (26.1% of population low variety versus 73.9% of the population with high variety).

Table 5-1 Food group description used for calculation of dietary variety scores

Food group	Number of food types	Food types
Starchy Foods	16	plain baby rice, flavoured baby rice, other cereals, gluten-free rusks, normal baby rusks, dried vegetable/pasta based meals, breakfast meals, vegetable/pasta/rice based meals, weetabix, other breakfast cereals, potatoes (boiled/mashed), rice, pasta, white bread/toast, brown bread/toast, crackers/breadsticks
Vegetables	7	carrots, other root vegetables, peas/green beans, green leafy vegetables, tinned tomatoes, other cooked vegetables, raw vegetables and salad
Fruit	5	pure fruit puree, cooked fruit, banana, oranges/satsumas/kiwis/strawberries, other fresh fruit
Dairy foods	5	milk/cereal based dessert , cheese, yoghurt/fromage frais, milk puddings, milk (excluding formula/breast milk)

Food group	Number of food types	Food types
Meat and protein foods	18	dried meat based meals, dried fish based meals, meat based meals, fish based meals, chicken/turkey, sausages/burgers, red meat (beef, lamb, pork), liver/kidney/heart, white fish, tuna, oily fish, eggs/quiche, baked beans, other beans/pulses, soya/ veggie burgers, Quorn, peanuts/peanut butter, other nuts
Total	51	

5.2.9 Covariates

Maternal age, education, and infants' gender and breastfeeding duration are significant predictors of fruit and vegetable preferences, infant feeding practices and dietary variety and therefore will be considered in the analysis.

5.2.10 Data analysis

Continuous data were checked for normality. Descriptive statistics are presented as means and standard deviations or 95% CI as appropriate. Non-normally distributed data are presented as medians and interquartile range. Descriptive statistics of categorical variables are presented as frequencies. The sample was stratified by age of solids introduction (dichotomised as before and after 6 months) and differences between the two groups are reported via independent samples t-tests or (where data was not normally distributed) the Mann Whitney U test. The level of significance was set at 0.05. Socio-demographic variables were obtained from data from other GO-CHILD questionnaires. Spearman's correlation was used to assess initial relationships between age of solids introduction (as continuous variable) and socioeconomic characteristics in order to build the models. Chi2-tests were used to analyse differences in feeding problems, taste preferences and dietary variety by age of solids introduction. In order to assess whether feeding problems, taste preferences and dietary variety were associated with age of solids introduction, odds ratios and 95% confidence intervals were calculated for each pair. Binary logistic regression was used to assess the relationship between introduction of solids before or after six months and the presence of reported feeding problems, meat preferences and high fruit or vegetable variety and overall dietary variety at 9 months. Unadjusted odds ratios and 95% CI were calculated using each outcome as the dependent variables and the age of solids introduction as the independent variable. Age of solids introduction was checked both as a binary and as a continuous variable for all outcomes, to reflect both the current recommendations cut-off as well as a potential dose response association. When used as a binary variable, the reference category was solids introduced before 6 months. Each available variable known to have an influence in the development of each outcome and age of solids introduction was then regressed individually. Two models were constructed where appropriate.

A statistical model (Model 1) where only variables that were found to be significant predictors or those with lower significance ($p<0.200$) were included and a conceptual model (Model 2) which consisted of all variables known in the literature as predictors of each outcome (regardless if they were not significantly correlated with age of solids introduction in the bivariate analyses in our sample).

Education was not included in the final models due to the large numbers of missing values which would have decreased the overall sample size of the model. However it was not a significant predictor in any of the bivariate models and its inclusion (data not shown) did not change the interpretation of any of the results.

Collinearity diagnostics were performed to exclude multicollinearity between the predictor variables in the regression models. Multicollinearity occurs when there is high correlation between independent variables. The existence of multicollinearity could result in very high standard errors and therefore less trustworthy b coefficients in the model. Moreover, adding 2 highly correlated variables in the model would limit the variance that these predictors account for, as the variance would be already explained by the first predictor, leaving the second predictor with only a small addition to the remaining variance (even though it may in reality account for a lot of the same variance). This could mean that it would be difficult to assess the individual contribution of each predictor (Field 2009). The first step to assess if multicollinearity was an issue in the data, was to examine if the independent variables used were highly correlated with each other. Additional colinearity tests, namely the calculation of variance inflation factor (VIF) and tolerance, were performed by regressing each predictor (used as a dependent variable) against all other independent variables and alternate until all variables were regressed against each other. A variance inflation factor of 10 or more indicates that there is a strong linear relationship between predictor variables. Tolerance is the inverse of VIF and a value of less than 0.1 indicates the presence of multicollinearity. There was no evidence of multi-collinearity between any of the independent variables in the GO-CHILD dataset.

5.2.11 Ethical Approval

The study received a favourable ethical approval from Eastern Scotland (Tayside) research ethics service. Study Number: 08/S1401/130

5.2.12 Funding

The GO-CHILD study is funded by the charity Sparks. Charity Number: 1003825

5.3 Results

By February 2012, 244 questionnaires were received, 230 of which from Sussex and 14 from Fife. For the purposes of this thesis, only the Sussex questionnaires were used in the analysis. Data on age of solids introduction was available for 210 infants of which 131 (62.4%) were introduced to solids before 6 months and 79 (37.6%) at or after 6 months.

5.3.1 Participants

The average mean age of the mothers in both groups was above 30 years, they were predominantly white and non-smoking, with the majority living in owned houses (Table 5-2). Mothers in the later complementary feeding group finished education later than mothers in the early weaned group and this was the only significant difference between the two groups (p=0.028). However it is worth noting that there were a lot of missing values in the education variable, due to unavailability of the dataset from the relevant questionnaire. Significant differences were seen in almost all infant characteristics; in the later weaned group there were more girls and as expected these infants were breast-fed for longer and were introduced to solids significantly later than the other group. However the average age of introduction of solids with lumpy texture did not differ between the two groups. The age of solids introduction was significantly associated with breastfeeding duration, age of introduction of lumpy solids and maternal age (Table 5-3). Although significant correlations were observed between duration of breastfeeding, age of lumps introduction and maternal age, the magnitude of the associations was small (rho coefficients ranging from 0.257-0.183), justifying their inclusion in the subsequent regression models without running the risk of multicollinearity. Tolerance values of less than 0.1 and VIF values of over 10 between all independent variables further confirmed that multicollinearity was not present in any of the regression models.

Table 5-2 Maternal and infant characteristics across the whole sample and stratified by age of solids introduction

	All		< 6 months		≥6 months		
	n	%	n	%	n	**%**	**P**
Maternal characteristics							
Age (years)[1]	212	33.8 (4.3)[1]	119	33.3 (4.4) [1]	73	34.1 (4.3) [1]	0.225[2]
Age finished education (years)[3]	80	19.5 (4)[3]	37	18 (4)[3]	34	21(3)[3]	**0.028[4]**
Age finished education	80		37		34		
18 years old or less	32	40	20	54.1	8	23.5	
19 years old or above	48	60	17	45.9	26	76.5	**0.009[5]**
Tenure	204		114		72		
Rent (%)	32	15.7	95	16.7	62	13.9	
Own (%)	172	84.3	19	83.3	10	86.1	0.611[5]
Smoking	203		113		72		
Yes (%)	3	1.5	2	1.8	0	0	
No (%)	200	98.5	111	98.2	72	100	0.256[5]
Smoked during pregnancy	203		113		72		
Yes (%)	10	4.9	7	6.2	2	2.8	
No (%)	193	95.1	106	93.8	70	97.2	0.292[5]

	All		< 6 months		≥6 months		
	n	%	n	%	n	**%**	**P**
Ethnicity	198		110		70		
White (%)	196	99	110	100	68	97.1	
Other (%)	2	1	0	0	2	2.9	0.150[6]
Infant characteristics							
Sex	224		129		75		
Male (%)	113	50.4	75	58.1	32	42.7	
Female (%)	111	49.6	54	41.9	43	57.3	**0.033[5]**
Breastfeeding duration (months)	211	8 (6)[3]	120	6 (6.6)[3]	75	9 (5)[3]	**0.050[4]**
Age of solids introduction (months)	210	5.4 (1.9)[3]	131	5(1.1)[3]	79	6 (0)[3]	**0.000[4]**
Age of introduction of lumps (months)[3]	223	7 (1)[3]	128	7 (1)[3]	76	7 (2)[3]	0.064[4]

[1] Mean (sd) ; [2]t-test; [3]median (interquartile range); [4] Mann Whitney test;[5] Chi square test; [6]Fisher's exact test

Table 5-3 Univariate associations (Spearman correlation coefficients) for potential predictor variables and age of solids introduction

Variables	Age of solids introduction	Breastfeeding duration	Age of lumps introduction	Maternal education
Breastfeeding duration (months)	**0.257****			
Age of lumps introduction (months)	**0.195****	-0.032		
Age mother finished education (years)	0.205	0.072	-0.026	
Maternal age (years)	**0.183***	**0.184***	0.189	0.013

Significant correlations are highlighted in bold. Significantly correlated **P<0.001, *p<0.05 (two-tailed).

5.3.2 Feeding practices

Most but not all infants were put on their mother's breast upon birth (213/229, 93%) and at 9 months 39.4% infants (86/218) were still being partially breast-fed. A small proportion of mothers reported adding cereal in the baby's bottle "always" (2/217, 0.9%), "often" (3/217, 1.4%), or "sometimes" (6/217, 2.8%). Very few mothers "sometimes" added sugar in their infants' foods or drinks (4/221, 1.8%). At 9 months 94.8% (218/230) of infants had been introduced to solids with a lumpy texture. In terms of using ready-made foods which are organic, over a third of the sample (85/230, 37%) reported that they use them "all the time", 26.5% (61/230) "most of the time", and 17% (39/230) "sometimes". Almost a fifth of the sample (42/230, 18.3%) stated that they do not use ready meals at all. In terms of drinking habits, 50.9% (108/212) reported that their infants drink usually from a cup, 35.4% (75/212) sometimes and 13.7% (29/212) reported that their baby does not drink from a cup at all. None of the infants were vegan at 9 months, however 5.1% (8/156) were following a vegetarian diet. The vast majority of the sample (128/153, 83.7%) reported that their infants would eat almost anything, whilst 14.4% (22/153) described their infants as "quite choosy" and 2% (3/153) as "very choosy".

5.3.3 Supplements

Approximately 9.1% (19/209) of infants were receiving vitamin or mineral supplements, of which 5 infants having Healthy Start vitamins, 4 infants receiving iron supplements, none of the infants consumed fluoride supplements and 10 infants were receiving other vitamins, such as multivitamin preparations, vitamin D and calcium tablets and omega 3 supplements. Consumption of oils (flax oil, hemp oil, linseed oil and olive oil) at 9 months was not very common with 5/155 mothers (3.2%) reporting consumption. Probiotics were consumed by 11.8% (18/153) of the sample.

5.3.4 Foods consumed

The types of foods consumed and well as the proportion of infants being offered these foods are shown in Table 5-4. There were significant differences in breast milk and formula milk consumption with all of the infants that were introduced to solids after 6 months being breast-fed (p=0.035) and infants introduced to solids before six months consuming higher amounts of formula (p<0.001). In terms of commercial infant foods, infants introduced to solids before 6 months were offered higher proportions of baby rice (p=0.014) and dried (0.008) and jarred vegetable/pasta (p=0.034) meals than those introduced to solids after six months.

Table 5-4 Proportion of infants being offered various foods by 9 months according to age at which they were introduced to solids

	Valid	**Consumers**	**Age of solids introduction**		
Food type	n	%	<6months	≥6months	**P***
Milk					
Breast milk	149	95.3	92.5	100	**0.035**
Ordinary formula	192	79.7	87.7	65.7	**<0.001**
Follow-on formula	166	69.3	74.5	60	0.051
Ordinary cow's milk	166	72.9	70.6	76.6	0.399
Goat's/sheep's milk	129	3.9	3.8	4.1	0.925
Hypo-allergenic formula	135	12.6	14.3	9.8	0.447

Food type	Valid n	Consumers %	Age of solids introduction <6months	 ≥6months	P*
Soya milk	95	11.6	15	5.7	0.172
Dry Infant foods					
Plain baby rice	204	82.8	88	74.7	**0.014**
Flavoured baby rice	196	27	28.5	24.7	0.563
Other cereals	203	89.2	89.6	88.5	0.800
Gluten free rusks	190	8.4	8.4	8.5	0.991
Normal baby rusks	201	42.8	46.4	36.8	0.184
Dried meat based meals	198	7.1	9	3.9	0.176
Dried fish based meals	198	1	0.8	1.3	0.734
Dried vegetable/pasta based meals	197	20.3	26.2	10.7	**0.008**
Dried desserts	195	3.1	4.1	1.4	0.286
Infant Jars/tins					
Breakfast meals	203	38.9	40.9	35.5	0.443
Meat based meals	209	67	72.5	57.7	0.027
Fish based meals	205	53.2	56.2	48	0.260
Vegetable/pasta/rice based meals	209	71.8	76.9	63.3	**0.034**
Milk/cereal based meal	207	69.1	69.5	68.4	0.859
Pure fruit puree	208	82.2	82.2	82.3	0.984
Other fruit desserts	187	36.4	36.5	36.1	0.955
Family foods					
Weetabix	206	66.5	67.7	64.6	0.640
Other breakfast cereals	201	41.3	38.1	46.7	0.233
Potatoes, boiled/mashed	206	95.1	96.9	92.1	0.121
Chips / roast potatoes	200	39	36.9	42.3	0.443
Carrots	210	99	99.2	98.7	0.716
Other root vegetables	207	98.1	97.7	98.7	0.584
Rice	205	72.7	70.6	75.9	0.406
Pasta	207	89.4	89.8	88.6	0.779
Chicken/turkey	207	87.4	88.4	86.1	0.642
Sausages/burgers	204	32.8	34.1	30.8	0.620

Food type	Valid	Consumers	Age of solids introduction		P*
	n	%	<6months	≥6months	
Red meat (beef, lamb, pork)	208	78.4	80.6	74.7	0.313
Liver, kidney, heart etc.	203	5.4	7	2.7	0.185
White fish	208	78.4	79.2	76.9	0.696
Tuna	146	43.2	40.6	48	0.393
Oily fish	204	55.4	55.5	55.3	0.977
Eggs/quiche	208	63.9	64.6	62.8	0.794
Cheese	205	86.3	87.5	84.4	0.533
Baked beans	205	51.7	46.9	59.7	0.074
Other beans/pulses	203	64.5	64	65.4	0.841
Peas/green beans	208	89.9	87.7	93.6	0.172
Green leafy veg	208	94.7	96.2	92.3	0.230
Tinned tomatoes	150	54.7	54.7	54.5	0.982
Other cooked vegetables	198	87.9	86.8	89.6	0.551
Raw veg and salad	203	61.1	57.5	67.1	0.173
Yoghurt/fromage frais	207	92.3	94.6	88.5	0.111
Milk puddings	205	49.3	48.8	50	0.870
Cooked fruit	205	75.6	76.2	74.7	0.807
Banana	208	96.6	96.9	96.2	0.787
Oranges/satsumas/ kiwis/strawberries	208	73.6	71.3	77.2	0.349
Other fresh fruit	195	84.1	83.7	84.7	0.856
Soya/ veggie burgers	204	10.3	11.8	7.8	0.360
Quorn	205	6.8	7.8	5.2	0.472
Peanuts/Peanut butter	205	16.1	13.4	20.5	0.178
Other nuts	203	9.4	8	11.5	0.400
White bread/toast	207	72.5	66.7	82.1	**0.016**
Brown bread/toast	205	82.9	85.2	73.2	0.274
Crackers/breadsticks	207	65.7	63.4	69.7	0.351
Biscuits	207	50.2	47.7	54.4	0.344
Cakes/buns/pastries	206	20.9	19.5	23.1	0.544
Crisps	206	17.5	16.4	19.2	0.605
Chocolate	207	20.8	22.5	17.9	0.436
Sweets	204	1.5	1.6	1.3	0.874
Ice cream	152	28.3	27.1	30.4	0.666

Food type	Valid n	Consumers %	Age of solids introduction <6months	 ≥6months	P*
Drinks					
Baby fruit juice	207	18.4	20.9	14.1	0.219
Other fruit juice	206	23.3	26	19	0.248
Ribena/Hi-juice blackcurrant squash	203	1	0.8	1.3	0.724
Other squash	203	2.5	3.2	1.3	0.391
Ribena lite/ diet blackcurrant squash	203	2.5	2.4	2.6	0.923
Other low calorie squash	205	5.4	6.3	3.9	0.469
Fizzy drinks	205	0.5	0.8	0	0.437
Tea	204	2.9	3.1	2.6	0.821
Water	209	97.6	96.9	98.7	0.406

*Chi squared tests, comparing < 6 months with ≥ 6 months

5.3.5 Feeding problems

Feeding difficulties were reported by parents between 19-49% of infants. There were no statistically significant differences in the prevalence of feeding problems between the complementary feeding groups apart from overeating, whereby infants introduced to solids before six months were more likely to be reported to overeat than those introduced to solids after six months (p=0.017). Looking at odds ratios for each feeding problem, neither the unadjusted nor adjusted models were statistically significant for having any feeding difficulty, consuming insufficient amounts of food, refusing to eat the right food, being choosy with food and not being able to establish an eating routine. Infants who were introduced to solids before 6 months were over 3 times more likely to have overeaten as reported by parents, in the adjusted analysis. Breastfeeding duration was the only other significant predictor in the bivariate analysis (data not shown); however when we adjusted for it (Model 1), the association remained almost unchanged and still significant. However, adjusting for all the other demographic and infant feeding factors resulted in a non-significant association (Model 2) (Table 5-5).

Table 5-5 Unadjusted and adjusted odds ratios (OR) for eating behaviours as reported by parents at 9 months of age and associations with age of solids introduction

Eating behaviours (%yes)	**Valid**	**All**	**Age of solids introduction**		
	n	%	<6months	≥6months	**P***
Any feeding difficulty	151	39.1	38.5	40	0.860
OR unadjusted	151		0.94 (0.48, 1.85)	1.00	0.860
OR adjusted Model 2	124		1.01 (0.45, 2.25)	1.00	0.983
Insufficient amount of food	151	49	46.9	52.7	0.489
OR unadjusted	151		0.79 (0.41, 1.54)	1.00	0.489
OR adjusted Model 1	149		0.98 (0.49, 1.97)	1.00	0.952
OR adjusted Model 2	124		1.38 (0.62, 3.11)	1.00	0.432
Refused to eat the right food	151	28.5	25	34.5	0.211
OR unadjusted	151		0.63 (0.31, 1.30)	1.00	0.213
OR adjusted Model 1	149		0.75 (0.35, 1.58)	1.00	0.443
OR adjusted Model 2	124		0.94 (0.40, 2.22)	1.00	0.888
Been choosy with food	151	44.4	44.8	43.6	0.891
OR unadjusted	151		1.05 (0.54, 2.04)	1.00	0.891
OR adjusted Model 1	149		1.31 (0.65, 2.65)	1.00	0.449
OR adjusted Model 2	124		1.51 (0.67, 3.40)	1.00	0.318
Over eaten	151	19.2	25	9.1	**0.017**
OR unadjusted	151		3.33 (1.19, 9.33)	1.00	**0.022**
OR adjusted Model 1	140		3.10 (1.09, 8.88)	1.00	**0.035**
OR adjusted Model 2	124		2.25 (0.74, 6.84)	1.00	0.154
No feeding routine established	151	13.2	10.4	18.2	0.176
OR unadjusted	151		0.52 (0.20, 1.35)	1.00	0.180
OR adjusted Model 1	149		0.60 (0.23, 1.58)	1.00	0.302
OR adjusted Model 2	124		0.59(0.18, 1.98)	1.00	0.397

Model 1 adjusted for infant sex apart from the "Over-eaten" category where Model 1 was adjusted for breastfeeding duration. In the "Any feeding difficulty none of the variables examined in the univariate analysis were significant predictors and therefore Model 1 was omitted.
Model 2 adjusted for breastfeeding duration, age of introduction of lumps, maternal age and infant sex

5.3.6 Food preferences

Preferences of fruits, vegetables and meat were very high in this population of infants with parents reporting high proportions of liking in all categories. There were no statistically significant associations between age of solids introduction and fruit, vegetable or meat preferences. There was also lack of variability with regards to fruit and vegetable preferences whereas meat appeared to be disliked by a larger proportion of infants introduced to solids after six months even though the difference was not statistically significant (Table 5-6). Median introduction of red meat was 7 months (IQR 6,8) with infants introduced to solids before six months starting to eat meat at 6 months (IQR 6,7) and those introduced to solids after 6 months at 7 months (IQR 7,8).

Table 5-6 Differences between groups of infants introduced to solids before or after 6 months for vegetable, fruit and meat preferences as reported by parents at 9 months

	Valid	**All**	**Age of solids introduction**		
Preferences (% yes)	n	%	<6months	≥6months	**P***
Likes vegetables	152	98	97.9	98.2	0.899
Likes fruit	150	99.3	98.9	100	0.439
Likes meat	139	92.1	94.4	88	0.181

Looking at meat preference in more detail, none of the predictors examined individually was significantly associated with liking meat. In the 2 adjusted models using all socio-demographic and infant feeding variables (Model 2) and just those with the lowest p values (Model 1), age of solids introduction was still not a significant predictor of meat preferences in this population of 9 month old infants (Table 5-7).The same non-significant results were obtained for Models 1 and 2 when age of solids introduction was used as a continuous variable (data not shown).

Table 5-7 Bivariate associations between meat preferences, age of solids introduction and several other potential predictors and adjusted models of meat preferences and age of solids introduction.

		Meat Preferences (Likes Meat)				
	n	**B**	**Wald χ^2**	**OR**	**95% CI**	**P**
Bivariate analysis						
Infant feeding practices						
Age of solids introduction (months)	139	-0.289	0.640	0.75	0.37, 1.52	0.424
Age of solids introduction (< 6 months)	139	0.829	1.712	2.29	0.66, 7.93	0.191
Breastfeeding duration (months)	132	0.026	0.073	1.03	0.85, 1.24	0.787
Age introduced red meat (months)	115	-0.193	0.273	0.82	0.4, 1.7	0.601
Age of intro of lumps (months)	139	-0.483	2.221	0.62	0.33, 1.17	0.136
Socio-demographic factors						
Infant sex (male)	140	0.920	1.992	2.51	0.70, 9.00	0.158
Maternal Education (years)	31	-0.191	0.725	0.83	0.53, 1.28	0.395
Maternal education (≤18 years)	31	0.258	0.040	1.29	0.10, 16.04	0.841
Maternal Age (years)	127	-0.031	0.138	0.97	0.82, 1.14	0.710
Model 1						
Age of solids introduction (< 6 months)	135	0.415	0.367	1.51	0.40, 5.80	0.545
Model 2						
Age of solids introduction(< 6 months)	96	-0.155	0.016	0.86	0.08, 9.68	0.900

Variables defined as follows: Age of solids introduction in months (m), as well as in 2 groups (gr) (before and after 6 months):the age at which a food other than formula milk or breast milk was introduced for the first time, breastfeeding duration in months (m): the age at which the infants had their last breastfeed; Age of introduction of lumps in months (m): the age at which the first solids food with a lumpy texture was introduced; Maternal education in years as well as in 2 groups (gr) (before 18 years and 19 years or above): the age at which the mother finished full time education

Model 1 adjusted for sex and age of introduction of lumps

Model 2 adjusted for sex, age of introduction of lumps, breastfeeding duration, maternal age and age of meat introduction

5.3.7 Food variety

Overall the majority of infants had a high variety of vegetables, fruit and general diet. There were no significant differences in dietary variety between infants introduced to solids before or after six months as seen in Table 5-8.

Table 5-8 Differences between groups of infants introduced to solids before or after 6 months for vegetable, fruit and meat preferences as reported by parents at 9 months

	Valid	All	Age of solids introduction		
High Dietary variety (% yes)	n	n	<6months	≥6months	**P**
Vegetable variety	230	193	80.9	88.6	0.143
Fruit variety	230	170	71.8	77.2	0.383
Overall food variety	230	168	70.2	73.4	0.620

The age of solids introduction (in months) was a significant predictor of high vegetable in the unadjusted bivariate analysis. This was not however the case when age of solids introduction was used as a categorical variable (before or after 6 months) or indeed any other of the potential predictive variables. In the adjusted models, the significance of age of solids introduction was lost (Table 5-9).

Table 5-9 Bivariate associations between vegetable variety, age of solids introduction and several other potential predictors and adjusted models of vegetable variety and age of solids introduction.

		High Vegetable variety				
	n	**B**	**Wald χ^2**	**OR**	**95% CI**	**p**
Bivariate analysis						
Infant feeding practices						
Age of solids introduction (months)	210	0.427	4.199	0.53	1.02, 2.31	**0.040**
Age of solids introduction (<6 months)	210	-0.607	2.105	0.55	0.24. 1.24	0.147
Breastfeeding duration (months)	211	0.104	2.860	1.11	0.98, 1.25	0.091
Age of intro of lumps (months)	223	-0.126	0.517	0.88	0.63, 1.24	0.472

	n	High Vegetable variety				
		B	Wald χ^2	OR	95% CI	p
Socio-demographic factors						
Infant sex (male)	224	0.154	0.178	1.17	0.57, 2.38	0.673
Maternal Education (years)	80	-0.023	0.092	0.98	0.84, 1.14	0.762
Maternal education (≤18 years)	80	0.659	0.839	1.93	0.47, 7.92	0.360
Maternal Age (years)	212	-0.009	0.045	0.99	0.91, 1.08	0.833
Model 1						
Age of solids introduction (months)	195	0.359	2.132	1.43	0.88, 2.32	0.144
Model 2						
Age of solids introduction (months)	170	0.463	2.62	1.59	0.91, 2.78	0.104

Variables defined as follows: Age of solids introduction in months (m), as well as in 2 groups (gr) (before and after 6 months):the age at which a food other than formula milk or breast milk was introduced for the first time, breastfeeding duration in months (m): the age at which the infants had their last breastfeed; Age of introduction of lumps in months (m): the age at which the first solids food with a lumpy texture was introduced; Maternal education in years as well as in 2 groups (gr) (before 18 years and 19 years or above): the age at which the mother finished full time education
Model 1 adjusted for breastfeeding duration
Model 2 adjusted for sex, age of introduction of lumps, breastfeeding duration, maternal age

The age of solids introduction was not a significant predictor of fruit or overall food variety in all models (both adjusted and unadjusted). None of the potential predictors tested were found to be significant in both outcomes (Tables 5-10 and 5-11).

5.3.8 Post-hoc sample calculation

Post hoc power calculations showed that using an alpha value of 0.05 and 151 participants (in the unadjusted regression models), this study had 80% power to detect a moderate effect size of d = 0.22.

Table 5-10 Bivariate associations between fruit variety, age of solids introduction and several other potential predictors and adjusted models of fruit variety and age of solids introduction.

		High Fruit variety				
	n	B	Wald χ^2	OR	95% CI	p
Bivariate analysis						
Infant feeding practices						
Age of solids introduction (months)	210	0.080	0.217	1.08	0.77, 1.52	0.641
Age of solids introduction (<6 months)	210	-0.288	0.757	0.75	0.39, 1.43	0.384
Breastfeeding duration (months)	211	0.095	3.724	1.10	1.00, 1.21	0.054
Age of introduction of lumps (months)	223	-0.155	1.140	0.86	0.65,1.14	0.286
Socio-demographic factors						
Infant sex (male)	224	-0.069	0.051	0.93	0.51, 1.70	0.821
Maternal Education (years)	80	-0.091	1.483	0.91	0.79, 1.06	0.223
Maternal education (≤18 years)	80	-0.160	0.097	0.85	0.31, 2.34	0.756
Maternal Age (years)	212	-0.019	0.251	0.98	0.91, 1.06	0.616
Model 1						
Age of solids introduction (months)	195	-0.090	0.220	0.91	0.63, 1.33	0.639
Model 2						
Age of solids introduction (months)	170	-0.267	1.415	0.77	0.49, 1.19	0.234

Variables defined as follows: Age of solids introduction in months (m), as well as in 2 groups (gr) (before and after 6 months):the age at which a food other than formula milk or breast milk was introduced for the first time, breastfeeding duration in months (m): the age at which the infants had their last breastfeed; Age of introduction of lumps in months (m): the age at which the first solids food with a lumpy texture was introduced; Maternal education in years as well as in 2 groups (gr) (before 18 years and 19 years or above): the age at which the mother finished full time education

Model 1 adjusted for breastfeeding duration

Model 2 adjusted for sex, age of introduction of lumps, breastfeeding duration, maternal age

Table 5-11 Bivariate associations between overall food variety, age of solids introduction and several other potential predictors and adjusted models of food variety and age of solids introduction.

		High Overall food variety				
	n	**B**	**Wald χ^2**	**OR**	**95% CI**	**p**
Bivariate analysis						
Infant feeding practices						
Age of solids introduction (months)	210	0.091	0.295	1.10	0.79, 1.52	0.587
Age of solids introduction (<6 months)	210	-0.158	0.245	0.85	0.46, 1.59	0.620
Breastfeeding duration (months)	211	0.010	0.043	1.01	0.92, 1.11	0.836
Age of intro of lumps (months)	223	-0.218	2.241	0.80	0.60, 1.07	0.134
Socio-demographic factors						
Infant sex (male)	224	0.070	0.054	1.07	0.60, 1.93	0.817
Maternal Education (years)	80	-0.011	0.036	0.99	0.88, 1.11	0.850
Maternal education (≤18 years)	80	-0.236	0.191	0.79	0.27, 2.28	0.662
Maternal Age (years)	212	-0.046	1.504	0.96	0.89, 1.02	0.220
Model 1						
Age of solids introduction (months)	204	0.221	1.559	1.25	0.88, 1.76	0.212
Model 2						
Age of solids introduction (months)	170	0.012	0.003	1.01	0.66, 1.55	0.955

Variables defined as follows: Age of solids introduction in months (m), as well as in 2 groups (gr) (before and after 6 months):the age at which a food other than formula milk or breast milk was introduced for the first time, breastfeeding duration in months (m): the age at which the infants had their last breastfeed; Age of introduction of lumps in months (m): the age at which the first solids food with a lumpy texture was introduced; Maternal education in years as well as in 2 groups (gr) (before 18 years and 19 years or above): the age at which the mother finished full time education

Model 1 adjusted for age of introduction of lumps

Model 2 adjusted for sex, age of introduction of lumps, breastfeeding duration, maternal age

5.4 Discussion

5.4.1 Main findings

The aim of the study was to examine the association between the timing of solids introduction with respect to feeding problems, dietary variety and food preferences at 9 months of age. The study showed that in this, Sussex infant population, which was highly selected as part of a larger study, infants who were introduced to solids before six months are over 3 times more likely to overeat compared to infants who were introduced to solids after 6 months, after adjusting for breastfeeding duration although the relationship disappeared when adjusting for socio-demographic confounders. The age of solids introduction was not an important predictor of other feeding problems, or of fruit, vegetable and overall food variety and meat preferences at 9 months, after adjusting for breastfeeding duration, age of introduction of lumpy solids, sex and maternal age.

5.4.2 Description of sample characteristics

Maternal characteristics across the whole sample were that of a predominantly white, older, non-smoking population with a high proportion of house ownership which suggests that the sample is drawn from a higher socioeconomic class. This is also reflected in the infants' characteristics as in both groups infants were breast-fed for longer and were introduced to solids later compared to the national infant feeding survey (McAndrew et al. 2012b)as well as other studies in the UK (Wright et al. 2004; Fewtrell et al. 2003; Anderson et al. 2001). Across the sample, 93% of infants were breast-fed at birth with almost 40% breastfeeding still at 9 months. Caton and colleagues (2011) found that mothers who breastfeed their infants irrespective of exclusivity or duration tended to introduce solids later than mothers who fed formula to their infants (22 weeks versus 18 weeks). Therefore even though there were some significant differences between the two groups in terms of some socio-demographic characteristics, in real terms the whole sample is that of a fairly homogenous, higher socioeconomic class population and therefore results need to be interpreted under this light. As expected, infants who were introduced to solids after 6 months had all been breast-fed and a significantly lower proportion had had infant formula compared to those introduced to solids later. Consumption of family foods was high in both groups and use of organic infant foods was also very popular, further confirming the nature of the sample.

5.4.3 Feeding problems

Approximately 25-40% of caregivers report feeding problems in their infants and toddlers in another study (Reau et al. 1996) and our results are in line with this observation. Due to the cross-sectional design of the study and the absence of concurrent growth rate data it was not possible to establish how severe or persistent these issues were and if they had an impact on the infants' growth and development.

None of the feeding problems were predicted by age of solids introduction either as a categorical variable (to reflect the current infant feeding recommendations) or as a continuous variable (to detect any dose-response association), before or after adjustment for confounding factors. A possible reason for this could be that because the median ages of solids introduction between the two groups were quite close, the difference was not large enough to reach statistical significance in the regression models. Some feeding problems, such as neophobia and fussy eating are also heritable and it is yet not known the extent to which genetics influence the development and severity of feeding difficulties (Leathwood & Maier 2005). This may potentially be true for other feeding difficulties as well. However there may be an interaction between genetic and environmental factors; for example parents who are themselves neophobic may provide a narrow range of foods to their infants (Blissett & Fogel 2013). We were not able to assess parental feeding problems so we were not able to establish their contribution to the infants' feeding difficulties.

The picture about overeating seems to be clearer; a much higher proportion of parents of infants introduced to solids before six months reported that their child had overeaten. In the statistically constructed model where duration of breastfeeding was the only other factor significantly associated with overeating, timing of solids introduction remained significant. As discussed in the previous chapter, exclusivity and duration of breastfeeding may be protective against obesity later in life (J A Scott et al. 2012; Hunsberger et al. 2013) and may facilitate better appetite regulation (Disantis et al. 2011; Robinson et al. 2013). However the fact that the relationship remained significant after accounting for t breastfeeding duration, suggests a possible independent association with the timing of solids introduction per se. Caton and colleagues found that mothers who introduced solids early to their infants worried less about their child becoming overweight but were concerned about their child's hunger whilst recognising their fullness cues, in other words whilst acknowledging their infant may be overeating being unable to reduce their intake (Caton et al. 2011). None of the other characteristics were significant however in the theoretical model when socio-demographics were included the relationship disappeared. It could be that due to the homogeneity of the sample or small size of sample these did not reach significance.

5.4.4 Food preferences

Surprisingly there was no variation in fruit or vegetable preferences between the complementary feeding groups and only 3-4 parents reported infants not liking these foods. Early feeding practices such as breastfeeding duration and early introduction of fruit and vegetables have been shown to be associated with children's intakes (Cooke et al. 2004), however we were not able to test this hypothesis due to the very small number of parents reporting fruit and vegetable dislikes. Children's preferences are a reflection of the foods that they are familiar with and availability of fruits, vegetables at home seems to be an important predictor of preferences and intake (Savage et al. 2007). The direction of the association was that more infants liked fruit and vegetables particularly if they were introduced to solids later (although not significantly different than those introduced to solids before six months). It could be argued that as the population is a long-term breast-fed one with health aware highly educated mothers it is likely that fruit and vegetables were regularly consumed in the household and therefore infants were more exposed to them, be it through flavours in breast milk, or because of high exposure during weaning. It is known that parental modelling and repeated exposure are major contributors to fruit and vegetable acceptance (Savage et al. 2007). Infants' first role model at the beginning of the complementary feeding period is their mother and the degree to which mothers consume fruit and vegetables or make those available to their infants will determine their acceptance and intake (Mennella & Ventura 2011). A study of 1291 children aged from 4 to 16 years in West London showed that the more familiar the food is, the more it is likely to be liked (Cooke & Wardle 2005). Parental consumption has been shown to be a highly significant predictor for fruit and vegetable intakes. Family feeding practices were also predictive of higher vegetable intake but not fruit (Cooke et al. 2004).

Even though we did not have access to the information on maternal diet, available for this analysis, we can assume that because of their demographics these mothers are likely to have a high intake of fruit and vegetables themselves and therefore the infants are likely to have been exposed to these foods during weaning. In a recent study of older infants from 3 countries (UK, Denmark, France), preferences for vegetables were linked to how many times they were offered them and in further analysis in the UK only, how often the mother consumed the vegetables herself (Ahern et al. 2013). In qualitative interviews, Caton et al.

noted that mothers reported changing their own eating habits to include more vegetables in order to provide a good example for their infants (Caton et al. 2011). The genetic element of food preferences should also not be overlooked. Parental similarities of food preferences to the young children are weak but positive and increase as the child grows older (Birch 1999). Therefore it can be argued that there are other factors that perhaps play a more important role in fruit and vegetable acceptance than age of solids introduction in this sample of infants.

Age of meat introduction was significantly different between the two groups of infants (6.6 versus 7.4 months); however it was not a significant predictive factor of meat preferences at 9 months. Meat was introduced later than other foods in the weaning process even in the group that started solids before six months and this was also evident in a study conducted in 2 European regions, one in Germany and France (median age of meat introduction 7 months in Germany and 6 months in France) (Maier et al. 2007). This is contrary to current infant feeding recommendations which advocate the introduction of meat of appropriate textures at the very beginning of weaning along with vegetables and fruit and starchy foods (British Dietetic Association 2013b). Meat liking and acceptance has not been previously looked into in great detail by many studies. As mentioned in the previous chapter, inclusion of highly bioavailable iron is very important in the early weaning period especially for infants who are introduced to solids after 6 months. However, when looking at meat preferences in more detail, none of the factors tested was associated with meat liking. A study by Schwartz and colleagues looked at the effect of duration of exclusive breastfeeding on different taste acceptance in infants at 6 and 12 months. They found that duration of exclusive breastfeeding was associated with positive acceptance for umami taste (which contains glutamate, an element present in meats and other protein foods as well as some vegetables) at 6 months but not at 12 months but just as in our findings, the age of solids introduction was not associated with umami acceptance (Schwartz et al. 2012).

Similarly to fruit and vegetable preferences, it could be that there was still not enough variability in the sample to detect differences or that meat preferences are related more to maternal intakes or genetic predisposition. We know that children at the age of 2 to 3 years choose animal products and starchy foods and avoid vegetables; however there is high individual variability of their food choices which is probably related to previous food experiences (Nicklaus et al. 2005). This was not the case in our sample, where fruits and vegetables were more liked than meat. It is however important to explore how parents assess a child's preference to a particular food. It is well known that repeated exposure is key to food acceptance (Schwartz et al. 2009). Apart from increasing intakes of the repeated food it also increases acceptance for foods of similar taste and texture (Birch et al. 1998). Depending on the sensory properties of each food (some foods are more easily accepted than others), up to 8-10 exposures may be needed for food acceptance (Sullivan & Birch 1994; Birch et al. 1998). However in a study by Carruth and colleagues, only 6-9% of caregivers offered a new food 6-8 times. The majority of parents offered a novel; food 3-5 times before deciding that their child did not like it and 25% offered new foods only 1-2 times (Carruth et al. 2004). Therefore as we do not know the number of repeated exposures of meat we cannot be sure how accurate the parental report of dislike to meat was.

5.4.5 Dietary variety

Age of solids introduction was a significant predictor of vegetable variety in the unadjusted analysis however this association diminished after adjusting for confounding factors. Timing of solids introduction was not a significant predictor in the other two categories before or after adjustment. This finding is consistent with a recent study by Scott and colleagues (Scott et al. 2012) who looked at core food and fruit and vegetable variety in 2 year old children in Australia. However in that study breastfeeding duration was a significant predictor of variety before and after adjustment for confounders. Also maternal age and education were significantly related, something that was not the case for our study. One possible explanation for this is that at 2 years children were more likely to be neophobic (Dovey et al. 2008) and therefore differences in dietary variety would be more evident in that age group as opposed to our 9 month old sample. Children of older mothers had generally tried fewer non-core foods, and liked a smaller proportion of non-core foods, however maternal age was not significantly related to the proportion of vegetables and fruits liked or tried (Howard et al. 2012).

Early variety not just in flavour, but also texture and colours drives the acceptance of new foods as seen in a study by Gerrish and Mennella, whereby introducing a variety of vegetables facilitated the acceptance of a new food (chicken puree) (Gerrish & Mennella 2001) and this effect is further assisted by breastfeeding duration (Maier et al. 2008). Therefore the fact that a variety of fruit and vegetables were offered may have enhanced acceptance in other food groups and this could explain the high overall variety observed in both groups. In this study both groups were breast-fed for a long period of time which could mean that infants were exposed to a variety of flavours both through breast milk and through complementary feeding concurrently. In a longitudinal study examining food variety in 6-8 year old children in the United States, fruit variety was predicted by fruit variety in the first 2 years of life as well as breastfeeding duration (Skinner et al. 2002). Earlier introduction of solids has been related to children liking a greater proportion of non-core foods at age two (Howard et al. 2012), something we did not observe in our study population as there were no differences in non-core food consumption between the two groups.

As mentioned before, the lack of association with socioeconomic and other infant feeding predictors even if not explained by the homogeneity of the sample may be also because of the influence of other factors such as parental preferences, modelling or parenting styles. Skinner showed that vegetable variety in 6-8 year old children was predicted by maternal vegetable preferences (Skinner et al. 2002). This could be the result of a combination of food availability (if a variety of foods is made available to them they will eat a more varied diet) and parental modelling as infants tend to imitate the behaviour of parents so if the parent consumes a variety of different foods, the infant is more likely to do the same (Wardle & Cooke 2008). Parenting styles defined as the way the parents and child interact with respect to attitudes and behaviours may also play a part in shaping food habits. An authoritative parenting style, whose main components are setting rules whilst being responsive to the child's cues, has been shown to elicit healthy eating habits although there is still no evidence of causality (Schwartz et al. 2011). A study of 502 French mothers with children aged between 20-36 months looking at the effect of maternal feeding practices and reported feeding difficulties found that predictive factors of children's feeding problems included permissive style parenting, and strategies to avoid conflict during mealtimes such as preparing foods that are known to be liked by the child, which prevented exposure to a variety of foods (Rigal et al. 2012).

5.5 Limitations

The main strengths of this study are the relatively large sample size and the information on age of introduction of different types of solids foods. Fruit and vegetable variety were also assessed separately as opposed to combined in other studies. Also we were able to examine meat preferences which is not something routinely asked as most studies focus of fruit and vegetables. There are several limitations mainly due to the fact that the questionnaire was designed for the purpose of assessing a different outcome. We did not have available any information on parental eating behaviours, their food preferences, intakes, parenting styles and weight which could have contributed to the interpretation of some of the results. We also had a large proportion of the cohort with missing data on educational status. Due to the cross-sectional design of the study it was not possible to ascertain any potential long-term association between the timing of solids introduction and dietary variety, food preferences or feeding problems. In terms of the generalizability of the study findings, the characteristics of the study sample are those of a predominantly white, older and well educated group which may have different food habits than a less affluent population. Therefore it is likely that there has been a misestimation of fruit and vegetable preferences as well as overall dietary variety (evident from the fact that data was skewed towards the top end for all outcomes) as it is known that in populations with less privileged backgrounds dietary quality is lower (Burnier et al. 2011). Selection bias is very common and therefore the results need to be interpreted with this in mind.

5.6 Chapter Summary

In conclusion, age of solids introduction was not a significant predictor of food preferences, dietary variety and feeding difficulties in this population of 9 month old infants from an affluent socioeconomic background. More research needs to focus on other aspects of feeding and particularly at the role of parental feeding practices and attitudes towards food.

Chapter 6

General Discussion

6 General Discussion

6.1 Introduction

The contribution of the thesis in further developing scientific knowledge lies in the examination of differences in outcomes between groups of infants defined by their age at solids introduction using the 6 month cut off point as advocated by WHO, as opposed to 4-6 month cut off point used historically and in the majority of studies so far. In one study, growth, eating behaviours, diet and iron status have been examined with hands-on measurements and in another study eating behaviours, dietary variety and food preferences have been examined using questionnaires. Moreover, a detailed examination of the above parameters has been conducted in infants who followed baby-led weaning over a period of 12 months compared to those who were spoon-fed, which has not been previously studied. A non-invasive method for measuring haemoglobin levels in infants was also tested in a community setting (participants' homes). In this chapter the main findings will be summarised, the main methodological limitations will be discussed in more depth and implications for practice and further research will be outlined. There will be a description of the dissemination of the study findings so far and plans for further publications.

The overall aims of this thesis were to assess the association between the age of solids introduction and growth, eating behaviours, diet and iron status in infants introduced to solids before or after six months and those who followed either baby-led weaning or were spoon-fed. An additional aim was to validate a novel non-invasive haemoglobin measurement device against a heel prick haemoglobin measurement method. The thesis contributes to the existing literature in the following ways:

By following principally breast-fed infants for 12 months and examining differences in growth, eating behaviours, food and nutrient intakes and haemoglobin levels between those introduced to solids before and after 6 months i.e. those following as closely as possible the current infant feeding guidelines at an early and a later stage of the complementary feeding process as well as between those who followed baby-led weaning and those who were spoon-fed.

By using a novel non-invasive technique to measure haemoglobin levels in 18 month old infants in their homes compared to results to a standard heel prick measurements method.

By looking at the association between the age of solids introduction in a sample of mixed-fed infants and later eating behaviours, dietary variety and food preferences.

6.2 Overview of main findings

Infants in the Brighton's Breast-fed Babies study had growth patterns within the healthy range of the WHO growth standards throughout the follow up period. At 8 months, there was a significant positive association between weight and BMI-for-age z score with age of solids introduction with infants introduced to solids later being larger than those introduced to solids earlier; however the differences diminished by 18 months and no other differences were observed in any of the remaining growth indices. These findings are in agreement with studies looking at later introduction of solids (after 4-5 months) where there does not seem to be a significant association between earlier and later solids introduction in weight, length or fat and lean mass (Mehta, et al, 1998) or in skinfold thickness (Baird et al. 2008) especially after adjustment for other confounding factors (Lande 2005). An interesting finding was the fact that infants who followed BLW were significantly larger (although within the healthy range of growth) than those who were spoon-fed both at 8 and 18 months. Since there has only been one study looking at BMI in BLW infants but was based on self-reported measures, it is not possible to interpret the result within the context of the wider literature.

In terms of nutrient intakes, low intakes of calcium, zinc, iron and vitamin D were observed at 8 months, which have been identified as "problem nutrients" (Brown 2007; Foote & Marriott 2003) and agrees with a previous study of developed countries (Allen 2005). Infants who were introduced to solids after 6 months had significantly lower intakes of riboflavin, Vitamin B6, Vitamin B12 and selenium at 8 months than infants who were introduced to solids before 6 months however there were no differences in the proportion of infants below the RNI in any of the nutrients. Low intakes of these nutrient could reflect low intakes of meat and other rich sources of these vitamins and minerals as suggested in several other studies (Krebs 2007; Park et al. 2012) Infants who followed baby-led weaning had significantly higher intakes of polyunsaturated fatty acids and lower intakes of riboflavin, Vitamin D and Iodine than those who were spoon-fed. At 18 months, Vitamin D intakes were still very low across the whole sample; however iron intakes were close to the recommended intakes as were the majority of other nutrients across both groups that were examined. The issue of low Vitamin D is widely known to be a problem in developed countries and especially in breast-fed infants (Braegger et al. 2013). Recently the England Chief Medical Officer in her annual report called for provision of vitamin D supplements (as part to the healthy start scheme) should be offered to all infants and children under 5, highlighting the current low intakes across all levels of socioeconomic status.

Haemoglobin levels were within the healthy range at 8 and 18 months across the whole sample and no significant differences were observed neither between infants introduced to solids before or after six months nor between those who followed baby-led weaning or spoon feeding. This was consistent with other studies looking at haemoglobin levels and age of solids introduction (Dewey, et al 1998)as well as haemoglobin levels in infants of the same age in large UK studies (Lennox et al. 2013; Emond et al. 1996). Haemospect® was not successful in providing valid haemoglobin measurements in 18 month old toddlers when compared to a heel prick measurement method. This is in contrast with the very good results obtained for neonates (Rabe et al. 2005; Rabe et al. 2010) but could reflect differences in skin thickness between the two age groups which could have had an impact in the way the white light was reflected back to the device.

Infants in the Brighton's Breast-fed Babies Study who were introduced to solids at or after 6 months were reported to significantly under-eat when compared to infants introduced to solids before 6 months, although the growth measurements did not provide any evidence of compromised growth indices. This result was not mirrored in the GO-CHILD study when examining the same outcome, however, infants who were introduced to solids before 6 months were more likely to overeat compared to those introduced to solids after 6 months; the association remained significant, after adjustment for breastfeeding duration but not when adjusted for other confounding factors. No significant differences were observed in eating behaviours at 18 months between infants in either of the two sets of groups. Age of solids introduction was not a significant predictor of any other eating difficulties, food preferences or dietary variety in this population of 9 month old infants after adjusting for confounding factors, which is consistent with current literature (Scott, et al, 2012) and highlights the potential effect of other parameters such as parental preferences, availability and exposure of foods and parenting styles (Schwartz et al. 2009; Nicklaus 2011).

The aims of the Brighton's Breast-fed Babies study (Chapter 3) were to assess growth, diet, iron status and eating behaviours in a sample of principally breast-fed infants stratified by early and late solids introduction as well as by mode of complementary feeding. Due to the small sample size, the study was not adequately powered to explore the research questions in depth and therefore the aims for this study were not met. The same was true for the validation of the Haemospect® device (Chapter 4) as the sample was drawn from the same population as above. In contrast, the GO-CHILD study aims (Chapter 5) of assessing the association between the age of solids introduction and eating behaviours, food preferences and dietary variety were partially met; although the study was adequately powered to detect a small effect size, the homogeneity of the sample meant that the results were not representative of a general population.

The next section will explore these methodological limitations, compare them with similar issues from other studies and discuss their implications in the interpretation of the results.

6.3 Methodological limitations

The small sample size of the Brighton's Breast-fed Babies study meant that the likelihood of type II error was very high which reduced the power to detect significant associations. Hence, statistically non-significant results may in fact have been significant, given an adequate study sample size. Type I error, namely that a significant relationship may not be true cannot be excluded either (Christley 2010). The small sample size also meant that it was not possible to conduct regression analyses and control for confounding factors that could have influenced differences in growth, iron status, diet or eating behaviours between the infant groups. According to Green, the minimum sample size for testing individual predictors in regression analysis is 104+k (k: number of predictors) (Green 1991). Miles and Shevlin have also estimated sample sizes for detecting different levels of power for different effect sizes depending on the number of predictors. For example for detecting a small effect size, with 5 predictors, a sample size of over 500 would be required (Miles & Shevlin 2001). The combination of breastfeeding for six months and introducing solids at six months remains rare, but as it is currently the practice recommended by health professionals to parents, its investigation on health outcomes is an important priority. Identifying and recruiting sufficient numbers of these participants remains a challenge, even on a national level. In the recent Diet and Nutrition Survey of Infants and Young Children (n=2683) only 2 children were exclusively breast-fed beyond 4 months both introduced solids between 4-6 months (n=329) (Lennox et al. 2013).

A closer look in the recruitment strategy of the Brighton's Breast-fed Babies study provided some insights of why recruitment was not successful and how this could be improved in future studies. Planning the recruitment process involved getting in touch with the local breastfeeding co-ordinator in order to establish links with the health visitors in the area and get a better understanding of the Brighton and Hove infant population. Two issues arose from the meeting with the breastfeeding co-ordinator. The relatively low number of mothers who exclusively breastfeed for six months as well as probable reluctance to take part in a research study. Breastfeeding rates in Brighton and Hove are higher than the national average both in terms of initiation rates as well as drop off rates. Brighton is also considered to be a relatively affluent area consisting of health aware, highly educated professional couples and families. It was therefore felt that recruiting in the area although challenging, would still be feasible. It was thought that the best way to recruit would be to establish links with the local health visitors who could provide their clients with the promotional material for the study; potential participants would be more likely to take part in the study if the information came from a practitioner with whom they had a prior rapport. This approach was used in the recruitment of participants for the GO-CHILD study by their research team and proved successful. However, when local health visitors were approached they felt that it would be quite time-consuming on their behalf and due to the fact that they were already understaffed and their services over-stretched they were not able to assist with recruitment. Therefore recruitment consisted of advertisements in local GP surgeries, pharmacies and the local press, and personal visits to breastfeeding drop-ins and children's clinics. Of these approaches the most successful ones were the face to face visits in clinics and breastfeeding drop ins which also led to snowballing. The main advantage for recruiting on the spot was that the potential participants had the chance to meet the researcher and develop a rapport prior to the home visits. Therefore the participants were reassured about the credentials of the researcher and were more likely to ask questions and get clarifications for the various stages of the study. The same principle was behind the success of snowballing. Participants who had already started the study were able to talk to their colleagues/friends about the experience and encourage them to take part. In a study such as this one which involved infants, invasive procedures and home visits, the building of trust between the researcher and the potential participants during the recruitment phase was vital.

One of the other potential barriers to recruitment was timing. Recruitment was conducted mostly during autumn and winter months where there were considerably less numbers of potential participants attending group sessions or even going out. In this particular case, new mothers with young babies of 5-6 months of age were less likely to be out in colder conditions. The situation became more challenging as the swine flu epidemic made potential participants even more reluctant not only to take their infants out but also to allow a person into their homes. December and January were particularly bad months for recruitment as the snowfall meant that most of the breastfeeding drop-ins and clinics were either cancelled or the participants could not reach them due to the adverse weather conditions. In breastfeeding drop-ins and clinics the main challenge was the fact that most new mothers came to discuss a feeding problem or other issues relating to breastfeeding their young babies. Therefore they were already pre-occupied and concerned with that and it was difficult to get their full attention and talk to them about the study. Also a lot of them were sleep deprived, tired and stressed dealing with a new baby so they were less likely to be in a position to commit to the study. The invasive nature of the study may have also contributed to the reluctance of some of the potential participants to get involved. Even though it was stressed in the participant information sheet and when recruiting in person that the blood collection was not compulsory, mothers were very sceptical and did not want to cause discomfort to their infants by allowing a heel prick.

Because of the small number of the participants recruited it was essential that losses to follow-up were minimised. It is known that the longer the follow-up period, the more likely it is that participants will be lost. In order to minimise loses to follow up, a number of measures were put in place. Firstly for each participant there were at least 3 methods of communication along with a postal address: a landline phone number, an email address and a mobile phone number. This ensured that there were alternative ways of contact, should the participant move house for example in the meantime. At each home visit every participant was also reminded to inform the researcher of any change of address. The majority of participants were recruited from the breastfeeding drop-ins so during the recruitment period the researcher saw them regularly during the drop-ins and thus kept in touch on a regular basis. The main challenge in the follow-up period was the interval between the second and third visits (10 months). Contact was still maintained with all participants through birthday cards; Christmas wishes emails and a few participants also contacted the researcher directly to arrange the last home visit or to inform her of changes in their address. These strategies maximised the study retention rate.

In summary, although the number of participants recruited was small, valuable lessons were learnt about the recruitment strategies that were more effective in this population group. The most important conclusion was that it was necessary to develop closer relationships with local healthcare professionals who had direct access to a large number of infants and who had already formed a good rapport with the mothers and their infants. Also, regular contact in between the study visits, ensured that loss to follow up was minimised.

Generalizability of results from both studies was the second methodological limitation of the thesis. The sample of mothers were more likely to be older in age, more highly educated, home owners, non-smoking and from a white ethnic background than the general population of mothers and therefore results could not be generalised beyond an affluent population. Selection bias is not uncommon in infant feeding research; it is known that mothers who decide to breastfeed longer and introduce solids later to their infants come from a higher socioeconomic backgrounds (McAndrew et al. 2012a). Baby-led weaning is also a practice more commonly found in samples with similar characteristics (Cameron et al. 2012). Although these results could not be applied to a population from a lower socioeconomic background, the infant feeding practices described represent the ideal for the current feeding recommendations and just like the WHO growth standards, a target that all mothers of infants, irrespective of their socio-demographic background should be aiming for.

6.4 Implications for research and practice

Even though firm conclusions on growth, diet and iron status were not able to be drawn due to the small sample size of the cohort, mothers of infants who are principally breast-fed for over 5 months as in this study, would benefit from the continued guidance and support from health professionals (health visitors, dieticians and paediatricians) especially in the first few months of the transition from breast milk to solids. Current guidance about raising awareness of the importance of iron after 6 months of age and encouragement to introduce rich sources of bioavailable iron as one of the first foods needs to be reiterated. Vitamin D supplements should also be encouraged and further attention needs to be drawn to the fact that organic products are not fortified with additional vitamins and minerals and therefore if mothers choose to use mostly organic products they need to ensure that a wide variety of foods is consumed to ensure that the infant is receiving adequate amounts of all nutrients. In terms of baby-led weaning, health professionals need to acknowledge its growing popularity and inform parents that there is still not enough evidence for or against the practice. In this study there was no evidence of growth faltering, anaemia or adverse eating behaviours in infants who were following baby-led weaning. However the above infant feeding advice would benefit these infants as well and ensure that they are receiving sufficient nutrients to fulfil their requirements for growth and development.

The GO-CHILD study, due to its larger sample size, provided more robust evidence of the association between the age of solids introduction and eating behaviours, food preferences and dietary variety. The evidence suggested that in terms of eating behaviours, preferences and dietary variety, the age of solids introduction was not a significant predictor. Health professionals could therefore stress the importance of parents as role models for shaping children’s preferences are well as the impact of effective parenting strategies in providing a variety of foods to infants.

6.5 Recommendations for future research

Replicating the Brighton's Breast-fed Babies Study with an adequate sample size would provide more robust evidence on the association between the age of solids introduction and health outcomes. A multicentre study would be an option worth considering in order to recruit sufficient numbers of participants following the current infant recommendations. Links with local health professionals and breastfeeding groups should also be considered as well as the use of social media to maximise recruitment rates. Iron intakes may be more usefully studied in conjunction with bioavailability, perhaps by examining when rich sources of iron are consumed together with iron enhancers or inhibitors. Iron status needs to be looked at using more than one indicator (such as ferritin and serum transferrin) and taking into consideration iron endowment from birth by looking at maternal iron status before and during pregnancy as well as the timing of umbilical cord clamping. Modifications to the Haemospect® device could be made and tested in a larger sample in order to access its effectiveness in measuring haemoglobin levels. In terms of eating behaviours, building from the evidence from the GO-CHILD study, further research should focus on other potential aspects that may play an important role such as parental food preferences, parenting styles and attitudes towards food, as well as parental BMI.

6.6 Dissemination of findings

The findings from this thesis have been disseminated to a variety of audiences through written and oral communications. A poster on the preliminary results of the Brighton's Breast-fed Babies Study was presented at the University of Brighton "Postgraduate Annual Research Presentation (PARP) day" in July 2010 and was awarded first prize. Delegates included other postgraduate students from the School of Pharmacy and Biomolecular Sciences, as well as lecturers and researchers from other departments of the Faculty of Science and Engineering. In December 2010, a poster of the same study was presented at the "Research Symposium for Dietitians new to research" at the Institute of Child Health in London which led to a subsequent publication of the abstract in the Journal of Human Nutrition and Dietetics (Ntouva A., Rogers I., MacAdam A. 2011). We submitted a rapid response to the article by Fewtrell et al. in the British Medical Journal (BMJ) " Six months of exclusive breast feeding: how good is the evidence?" in February 2011 which was posted on the BMJ website (Ntouva, A., Emmett, P., MacAdam, A., Rabe, H., Ranganathan, S., Williams, C. and Rogers 2011) [2]. We were invited to give a talk to neonatal nurses on the 8 month results of the Brighton's Breast-fed Babies Study at the "Neonatal Research Seminar" at the Royal Sussex County Hospital in May 2011. We presented a poster on the Brighton's Breast-fed Babies Study data at the international conference "Nutrition and Nurture in Infancy and Childhood" in Grange Over Sands, Cumbria, organized by the Maternal and Infant Nutrition and Nurture Unit (MAINN), School of Health, University of Central Lancashire in June 2011. The audience included midwives, health visitors, public health nutritionists, policy makers, researchers and distinguished academics in the field of infant feeding. We won 2nd place in a poster competition at the fifth "annual Surrey, Sussex and Kent Regional Paediatric and Neonatal Research Network Day" in conjunction with the Reproductive Health & Childbirth Local Specialty Group held at the Audrey Emerton Education Centre, Brighton. We presented a poster on the GO-CHILD Study data at the international conference "Nutrition and Nurture in Infancy and Childhood" in Grange Over Sands, Cumbria, in June 2013 the abstract of which will be published in the Journal of Maternal and Child Nutrition. An oral presentation entitled "Age of solids introduction, diet and food preferences in 9 month old infants" was given at the University of Brighton's Doctoral College "Research Student Conference: Making Science accessible". A manuscript entitled "Iron intakes and haemoglobin levels among breast-fed infants introduced to solids at or around six months" is currently under preparation and another journal paper based on GO-CHILD data will be drafted in the coming months.

6.7 Conclusion

Timing of solids introduction was not found to be an important predictor of food preferences, dietary variety and eating behaviours at 9 months. In the Haemospect® study, the non-invasive technique was not successful in measuring accurately haemoglobin levels at 18 months. The Brighton's Breast-fed Babies Studies provided a unique opportunity to explore the feasibility of conducting a longitudinal study with infants who follow the current feeding recommendations. Even though firm conclusions on the association between the age of solids introduction and growth, diet and iron status could not be drawn from this small sample of infants, useful insights were gained with respect to the recruitment challenges in this area of research and recommendations for the design of

[2] Available at: http://www.bmj.com/rapid-response/2011/11/03/very-low-dietary-iron-intakes-normal-haemoglobin-levels-8-months-among-ful (Assessed July 2013)

future studies were made. It is hoped that the insights gained into the challenges faced during these studies will assist in the successful design of future studies with similar population groups.

References

7 References

Agostoni, C., Decsi, T., Fewtrell, M., Goulet, O., Kolacek, S., Koletzko, B., Michaelsen, K.F., Moreno, L., Puntis, J., Rigo, J., Shamir, R., Szajewska, H., Turck, D. & van Goudoever, J., 2008. Complementary feeding: a commentary by the ESPGHAN Committee on Nutrition. *Journal of pediatric gastroenterology and nutrition*, 46(1), pp.99–110.

Ahern, S.M., Caton, S.J., Bouhlal, S., Hausner, H., Olsen, A., Nicklaus, S., Møller, P. & Hetherington, M.M., 2013. Eating a rainbow. Introducing vegetables in the first years of life in 3 European countries. *Appetite*, 71, pp.48–56.

Allen, L.H., 2012. B vitamins in breast milk: relative importance of maternal status and intake, and effects on infant status and function. *Advances in nutrition (Bethesda, Md.)*, 3(3), pp.362–9.

Allen, L.H., 2005. Multiple micronutrients in pregnancy and lactation: an overview. *Am J Clin Nutr*, 81(Suppl), p.1206S–1212S.

Anderson, A.S., Guthrie, C.A., Alder, E.M., Forsyth, S., Howie, P.W. & Williams, F.L., 2001. Rattling the plate--reasons and rationales for early weaning. *Health education research*, 16(4), pp.471–9.

Anderson, J., Malley, K. & Snell, R., 2009. Is 6 months still the best for exclusive breastfeeding and introduction of solids? A literature review with consideration to the risk of the development of allergies. *Breastfeeding review : professional publication of the Nursing Mothers' Association of Australia*, 17(2), pp.23–31.

Arden, M.A., 2010. Conflicting influences on UK mothers' decisions to introduce solid foods to their infants. *Matern Child Nutr*, 6(2), pp.159–173.

Baird, J., Poole, J., Robinson, S., Marriott, L., Godfrey, K., Cooper, C., Inskip, H. & Law, C., 2008. Milk feeding and dietary patterns predict weight and fat gains in infancy. *Paediatr Perinat Epidemiol*, 22(6), pp.575–586.

Baker, J.L., Michaelsen, K.F., Rasmussen, K.M. & Sørensen, T.I. a, 2004. Maternal prepregnant body mass index, duration of breastfeeding, and timing of complementary food introduction are associated with infant weight gain. *The American journal of clinical nutrition*, 80(6), pp.1579–88.

Baker, R.D., Greer, F.R. & Committee on Nutrition, 2010. Clinical Report-Diagnosis and Prevention of Iron Deficiency and Iron-Deficiency Anemia in Infants and Young Children (0-3 Years of Age). *Pediatrics*, 126(5), pp.1040–1050.

Bamberg, R., 2008. Occurrence and detection of iron-deficiency anemia in infants and toddlers. *Clin Lab Sci*, 21(4), pp.225–231.

Barker, S.J. & Badal, J.J., 2008. The measurement of dyshemoglobins and total hemoglobin by pulse oximetry. *Current opinion in anaesthesiology*, 21(6), pp.805–10.

Batal, M., Boulghourjian, C. & Akik, C., 2010. Complementary feeding patterns in a developing country: a cross-sectional study across Lebanon/Alimentation de complément dans un pays en voie de développement : une étude transversale au Liban. *Eastern Mediterranean Health Journal*, 16(2), pp.180–186.

Bates, B., Lennox, A., Prentice, A., Bates, C. & Swan, G., 2012. *National diet and nutrition survey: Headline results from Years 1, 2 and 3 (combined) of the Rolling Programme (2008/2009 – 2010/11)*, Department of Health / Food Standards Agency.

Beard, J. & Han, O., 2009. Systemic iron status. *Biochimica et biophysica acta*, 1790(7), pp.584–8.

Becquet, R., Leroy, V., Ekouevi, D.K., Viho, I., Castetbon, K., Fassinou, P., Dabis, F., Timite-Konan, M. & Study, A. 1201-1202 D.P., 2006. Complementary feeding adequacy in relation to nutritional status among early weaned breastfed children who are born to HIV-infected mothers: ANRS 1201/1202 Ditrame Plus, Abidjan, Cote d'Ivoire. *Pediatrics*, 117(4), pp.e701–10.

Belfort, M.B. & Gillman, M.W., 2013. Healthy infant growth: what are the trade-offs in the developed world?. *Nestlé Nutrition Institute workshop series*, 71, pp.171–84.

Bingham, A., Gill, C., Welch, A., Day, K., Cassidy, A., Khaw, K., Sneyd, M., Key, A., Roe, L. & Day, N., 1994. Comparison of dietary assessment methods in nutritional epidemiology : weighed records v. 24 h recalls, food-frequency questionnaires and estimated-diet records. *British Journal of Nutrition*, 72, pp.619–643.

Birch, L., 1999. Development of food preferences. *Annual review of nutrition*, 19, pp.41–62.

Birch, L., Savage, J.S. & Ventura, A., 2007. Influences on the Development of Children ' s Eating Behaviours : From Infancy to adolescence. *Canadian Journal of Dietetic Practice and Research*, 68(1), pp.s1–s56.

Birch, L.L., Gunder, L., Grimm-Thomas, K. & Laing, D.G., 1998. Infants' consumption of a new food enhances acceptance of similar foods. *Appetite*, 30(3), pp.283–95.

Bland, J. & Altman, D., 1999. Measuring agreement in method comparison studies. *Statistical methods in medical research*, 8, pp.135–160.

Blissett, J. & Fogel, A., 2013. Intrinsic and extrinsic influences on children's acceptance of new foods. *Physiology & behavior*, 121, pp.89–95.

Blossfeld, I., Collins, a., Kiely, M. & Delahunty, C., 2007. Texture preferences of 12-month-old infants and the role of early experiences. *Food Quality and Preference*, 18(2), pp.396–404.

Braegger, C., Campoy, C., Colomb, V., Decsi, T., Domellof, M., Fewtrell, M., Hojsak, I., Mihatsch, W., Molgaard, C., Shamir, R., Turck, D. & van Goudoever, J., 2013. Vitamin D in the healthy European paediatric population. *Journal of pediatric gastroenterology and nutrition*, 56(6), pp.692–701.

British Dietetic Association, 2013a. *Complementary Feeding: Introduction of solid food to an Infants Diet- Policy Statement*, The British Dietetic Association.

British Dietetic Association, 2010. *Practical Dietary Prevention Strategies for Infants at risk of developing Allergic Diseases - Consensus Statement*, The British Dietetic Association.

British Dietetic Association, 2013b. *Weaning your child - Fact Sheet*, The British Dietetic Association.

Brown, A. & Lee, M., 2011a. A descriptive study investigating the use and nature of baby-led weaning in a UK sample of mothers. *Matern Child Nutr*, 7(1), pp.34–47.

Brown, A. & Lee, M., 2013. An exploration of experiences of mothers following a baby-led weaning style: developmental readiness for complementary foods. *Matern Child Nutr*, 9(2), pp.233–243.

Brown, A. & Lee, M., 2011b. Maternal control of child feeding during the weaning period: differences between mothers following a baby-led or standard weaning approach. *Maternal and child health journal*, 15(8), pp.1265–71.

Brown, K.H., 2007. Breastfeeding and complementary feeding of children up to 2 years of age. *Nestlé Nutrition workshop series. Paediatric programme*, 60, pp.1–10; discussion 10–3.

Burdette, H.L., Whitaker, R.C., Hall, W.C. & Daniels, S.R., 2006. Breastfeeding, introduction of complementary foods, and adiposity at 5 y of age. *Am J Clin Nutr*, 83(3), pp.550–558.

Burnier, D., Dubois, L. & Girard, M., 2011. Exclusive breastfeeding duration and later intake of vegetables in preschool children. *Eur J Clin Nutr*, 65(2), pp.196–202.

Butte, N., Lopez-Alarcon, M. & Garza, C., 2002. *Nutrient adequacy of exclusive breastfeeding for the term infant during the first six months of life*, Geneva: World Health Organisation.

Butte, N.F., 2009. Impact of infant feeding practices on childhood obesity. *The Journal of nutrition*, 139(2), p.412S–6S.

Butte, N.F., Wong, W.W., Hopkinson, J.M., Smith, E.O. & Ellis, K.J., 2000. Infant feeding mode affects early growth and body composition. *Pediatrics*, 106(6), pp.1355–1366.

Cameron, S.L., Heath, A.-L.M. & Taylor, R.W., 2012. How feasible is Baby-led Weaning as an approach to infant feeding? A review of the evidence. *Nutrients*, 4(11), pp.1575–609.

Carruth, B.R., Ziegler, P.J., Gordon, A. & Barr, S.I., 2004. Prevalence of picky eaters among infants and toddlers and their caregivers' decisions about offering a new food. *J Am Diet Assoc*, 104(Suppl 1), pp.s57–64.

Caton, S.J., Ahern, S.M. & Hetherington, M.M., 2011. Vegetables by stealth. An exploratory study investigating the introduction of vegetables in the weaning period. *Appetite*, 57(3), pp.816–25.

Cattaneo, A., Williams, C., Pallas-Alonso, C.R., Hernandez-Aguilar, M.T., Lasarte-Velillas, J.J., Landa-Rivera, L., Rouw, E., Pina, M., Volta, A. & Oudesluys-Murphy, A.M., 2011. ESPGHAN's 2008 recommendation for early introduction of complementary foods: how good is the evidence? *Matern Child Nutr*, 7(4), pp.335–343.

Chan, L., Magarey, A.M. & Daniels, L.A., 2011. Maternal feeding practices and feeding behaviors of Australian children aged 12-36 months. *Matern Child Health J*, 15(8), pp.1363–1371.

Chantry, C.J., Howard, C.R. & Auinger, P., 2007. Full breastfeeding duration and risk for iron deficiency in U.S. infants. *Breastfeeding medicine : the official journal of the Academy of Breastfeeding Medicine*, 2(2), pp.63–73.

Chaparro, C.M., Neufeld, L.M., Tena Alavez, G., Eguia-Liz Cedillo, R. & Dewey, K.G., 2006. Effect of timing of umbilical cord clamping on iron status in Mexican infants: a randomised controlled trial. *Lancet*, 367(9527), pp.1997–2004.

Chatoor, I., 2009. Sensory food aversions in infants and toddlers. *Zero to Three*, (January), pp.44–49.

Christley, R.M., 2010. Power and Error: Increased Risk of False Positive Results in Underpowered Studies. *The Open Epidemiology Journal*, 3, pp.16–19.

Chua, A.C., Graham, R.M., Trinder, D. & Olynyk, J.K., 2007. The regulation of cellular iron metabolism. *Crit Rev Clin Lab Sci*, 44(5-6), pp.413–459.

Cohen, R.J., Brown, K.H., Canahuati, J., Rivera, L.L. & Dewey, K.G., 1994. Effects of age of introduction of complementary foods on infant breast milk intake, total energy intake, and growth: a randomised intervention study in Honduras. *Lancet*, 344(8918), pp.288–293.

Committee on Medical Aspects of Food Policy (COMA), 1991. *Dietary Reference Values for Food Energy and Nutrients for the United. Kingdom*, London, United Kingdom, London: HSMO.

Cooke, L. & Fildes, a, 2011. The impact of flavour exposure in utero and during milk feeding on food acceptance at weaning and beyond. *Appetite*, 57(3), pp.808–11.

Cooke, L.J. & Wardle, J., 2005. Age and gender differences in children's food preferences. *British Journal of Nutrition*, 93(05), pp.741–746.

Cooke, L.J., Wardle, J., Gibson, E.L., Sapochnik, M., Sheiham, A. & Lawson, M., 2004. Demographic, familial and trait predictors of fruit and vegetable consumption by pre-school children. *Public health nutrition*, 7(2), pp.295–302.

Coulthard, H., Harris, G. & Emmett, P., 2009. Delayed introduction of lumpy foods to children during the complementary feeding period affects child's food acceptance and feeding at 7 years of age. *Matern Child Nutr*, 5(1), pp.75–85.

Coulthard, H., Harris, G. & Emmett, P., 2010. Long-term consequences of early fruit and vegetable feeding practices in the United Kingdom. *Public health nutrition*, 13(12), pp.2044–51.

Cowin, I. & Emmett, P., 2001. Diet in a group of 18-month-old children in South West England, and comparison with the results of a national survey. *Journal of Human Nutrition and Dietetics*, 20(3), pp.254–267.

Cowin, I., Emond, a & Emmett, P., 2001. Association between composition of the diet and haemoglobin and ferritin levels in 18-month-old children. *European journal of clinical nutrition*, 55(4), pp.278–86.

Cribb, V.L., Warren, J.M. & Emmett, P.M., 2012. Contribution of inappropriate complementary foods to the salt intake of 8-month-old infants. *European journal of clinical nutrition*, 66(1), pp.104–10.

Department of Health, 1994. *Weaning and the weaning diet*, London: HMSO.

Dewey, K. & Chaparro, C.M., 2007. Session 4: Mineral metabolism and body composition iron status of breast-fed infants. *Proc Nutr Soc*, 66(3), pp.412–422.

Dewey, K.G., 2006. What is the optimal age for introduction of complementary foods? *Nestle Nutr Workshop Ser Pediatr Program*, 58, pp.161–165.

Dewey, K.G. & Chaparro, C.M., 2007. Session 4: Mineral metabolism and body composition iron status of breast-fed infants. *Proc Nutr Soc*, 66(3), pp.412–422.

Dewey, K.G., Cohen, R.J., Rivera, L.L. & Brown, K.H., 1998. Effects of age of introduction of complementary foods on iron status of breast-fed infants in Honduras. *The American journal of clinical nutrition*, 67(5), pp.878–84.

Dewey, K.G., Heinig, M.J., Nommsen, L.A., Peerson, J.M. & Lonnerdal, B., 1993. Breast-fed infants are leaner than formula-fed infants at 1 y of age: the DARLING study. *The American journal of Clinical Nutrition*, 57(2), pp.140–145.

Dewey, K.G., Heinig, M.J., Nommsen, L.A., Peerson, J.M. & Lonnerdal, B., 1992. Growth of breast-fed and formula-fed infants from 0 to 18 months: the DARLING Study. *Pediatrics*, 89(6), pp.1035–1041.

Disantis, K.I., Collins, B.N., Fisher, J.O. & Davey, A., 2011. Do infants fed directly from the breast have improved appetite regulation and slower growth during early childhood compared with infants fed from a bottle? *Int J Behav Nutr Phys Act*, 8, pp.89–99.

Domellof, M., Dewey, K.G., Lonnerdal, B., Cohen, R.J. & Hernell, O., 2002. The diagnostic criteria for iron deficiency in infants should be reevaluated. *J Nutr*, 132(12), pp.3680–3686.

Domellof, M., Lind, T., Lonnerdal, B., Persson, L.A., Dewey, K.G. & Hernell, O., 2008. Effects of mode of oral iron administration on serum ferritin and haemoglobin in infants. *Acta Paediatr*, 97(8), pp.1055–1060.

Domellof, M., Lonnerdal, B., Abrams, S.A. & Hernell, O., 2002. Iron absorption in breast-fed infants: effects of age, iron status, iron supplements, and complementary foods. *Am J Clin Nutr*, 76(1), pp.198–204.

Dovey, T.M., Staples, P. a, Gibson, E.L. & Halford, J.C.G., 2008. Food neophobia and "picky/fussy" eating in children: a review. *Appetite*, 50(2-3), pp.181–93.

Dunn, W., 1997. The impact of sensory processing abilities on the daily lives of young children and their families: A conceptual model. *Infants & Young Children*, 9(4), pp.23–35.

Durmuş, B., van Rossem, L., Duijts, L., Arends, L.R., Raat, H., Moll, H. a, Hofman, A., Steegers, E. a P., Jaddoe, V.W. V & Durmus, B., 2011. Breast-feeding and growth in children until the age of 3 years: the Generation R Study. *Br J Nutr*, 105(11), pp.1704–1711.

Eck, L.H., Klesges, L.M. & Klesges, R.C., 1996. Precision and estimated accuracy of two short-term food frequency questionnaires compared with recalls and records. *Journal of clinical epidemiology*, 49(10), pp.1195–200.

Edison, E.S., Bajel, A. & Chandy, M., 2008. Iron homeostasis: new players, newer insights. *European journal of haematology*, 81(6), pp.411–24.

EFSA Panel on Dietetic Products, N. and A. (NDA), 2009. Scientific Opinion on the appropriate age for introduction of complementary feeding of infants. *EFSA Journal*, 7(12), p.1423.

Elmadfa, I. & Meyer, A.L., 2012. Vitamins for the first 1000 days: preparing for life. *International journal for vitamin and nutrition research. Internationale Zeitschrift für Vitamin- und Ernährungsforschung. Journal international de vitaminologie et de nutrition*, 82(5), pp.342–7.

Emmett, P., 2009. Dietary assessment in the Avon Longitudinal Study of Parents and Children. *European journal of clinical nutrition*, 63 Suppl 1, pp.S38–44.

Emmett, P., North, K. & Noble, S., 2000. Types of drinks consumed by infants at 4 and 8 months of age: a descriptive study. *Public Health Nutrition*, 3(02), pp.211–217.

Emond, a M., Hawkins, N., Pennock, C. & Golding, J., 1996. Haemoglobin and ferritin concentrations in infants at 8 months of age. *Archives of Disease in Childhood*, 74(1), pp.36–39.

Fanaro, S., Borsari, G. & Vigi, V., 2007. Complementary feeding practices in preterm infants: an observational study in a cohort of Italian infants. *J Pediatr Gastroenterol Nutr*, 45 (Suppl 3), pp.S210–S214.

Fewtrell, M., Wilson, D.C., Booth, I. & Lucas, A., 2011. Six months of exclusive breast feeding: how good is the evidence? *BMJ (Clinical research ed.)*, 342, p.c5955.

Fewtrell, M.S., Lucas, A. & Morgan, J.B., 2003. Factors associated with weaning in full term and preterm infants. *Archives of disease in childhood. Fetal and neonatal edition*, 88(4), pp.F296–F301.

Field, A., 2009. *Discovering statistics using SPSS* 3rd Editio., London: Sage Publications.

Fields, D.A., Gilchrist, J.M., Catalano, P.M., Giannì, M.L., Roggero, P.M. & Mosca, F., 2011. Longitudinal Body Composition Data in Exclusively Breast-Fed Infants: A Multicenter Study. *Obesity*, 19(9), pp.1887–1891.

Food Standards Agency, 2005. *Food Portion Sizes* 3rd ed., London: TSO.

Foote, K.D. & Marriott, L.D., 2003. Weaning of infants. *Archives of disease in childhood*, 88(6), pp.488–92.

Forestell, C.A. & Mennella, J.A., 2007. Early determinants of fruit and vegetable acceptance. *Pediatrics*, 120(6), pp.1247–54.

Gambling, L., Andersen, H.S. & McArdle, H.J., 2008. Iron and copper, and their interactions during development. *Biochem Soc Trans*, 36(6), pp.1258–1261.

Ganchrow, J., Steiner, J. & Daher, M., 1983. Neonatal facial expressions in response to different qualities and intensities of gustatory stimuli. *Infant Behavior and Development*, 4, pp.473–484.

Geissler, C. & Singh, M., 2011. Iron, meat and health. *Nutrients*, 3(3), pp.283–316.

Gerrish, C.J. & Mennella, J.A., 2001. Flavor variety enhances food acceptance in formula-fed infants. *The American journal of clinical nutrition*, 73(6), pp.1080–5.

Gil, A., Ramirez, M. & Gil, M., 2003. Role of long-chain polyunsaturated fatty acids in infant nutrition. *European journal of clinical nutrition*, 57(Suppl 1), pp.S31–4.

Glasson, C., Chapman, K. & James, E., 2011. Fruit and vegetables should be targeted separately in health promotion programmes: differences in consumption levels, barriers, knowledge and stages of readiness for change. *Public health nutrition*, 14(4), pp.694–701.

Grant, C.C., Wall, C.R., Brewster, D., Nicholson, R., Whitehall, J., Super, L. & Pitcher, L., 2007. Policy statement on iron deficiency in pre-school-aged children. *J Paediatr Child Health*, 43(7-8), pp.513–521.

Green, S.B., 1991. How Many Subjects Does It Take To Do A Regression Analysis. *Multivariate Behavioral Research*, 26(3), pp.499–510.

Grimshaw, K.E.C. et al., 2009. Infant feeding and allergy prevention: a review of current knowledge and recommendations. A EuroPrevall state of the art paper. *Allergy*, 64(10), pp.1407–16.

Grossman, X., Chaudhuri, J.H., Feldman-Winter, L. & Merewood, A., 2012. Neonatal weight loss at a US Baby-Friendly Hospital. *Journal of the Academy of Nutrition and Dietetics*, 112(3), pp.410–3.

Gunnarsson, B.S., Thorsdottir, I., Palsson, G. & Gretarsson, S.J., 2007. Iron status at 1 and 6 years versus developmental scores at 6 years in a well-nourished affluent population. *Acta Paediatr*, 96(3), pp.391–395.

Harris, G., 2008. Development of taste and food preferences in children. *Current opinion in clinical nutrition and metabolic care*, 11(3), pp.315–9.

Harris, G., Thomas, A. & Booth, D.A., 1990. Development of salt taste in infancy. *Developmental Psychology*, 26(4), pp.534–538.

Haschke, F. & van't Hof, M.A., 2000. Euro-Growth references for breast-fed boys and girls: influence of breast-feeding and solids on growth until 36 months of age. Euro-Growth Study Group. *J Pediatr Gastroenterol Nutr*, 31 Suppl 1, pp.S60–71.

Heinig, M. & Nommsen, L., 1993. Intake and growth of breast-fed and formula-fed infants in relation to the timing of introduction of complementary foods: the DARLING study. *Acta Paediatrica*, 82, pp.999–1006.

Heird, W.C., Ziegler, P., Reidy, K. & Briefel, R., 2006. Current electrolyte intakes of infants and toddlers. *Journal of the American Dietetic Association*, 106(Suppl 1), pp.S43–51.

Hopkins, D., Emmett, P., Steer, C., Rogers, I., Noble, S. & Emond, A., 2007. Infant feeding in the second 6 months of life related to iron status: an observational study. *Archives of disease in childhood*, 92(10), pp.850–4.

Howard, A.J., Mallan, K.M., Byrne, R., Magarey, A. & Daniels, L.A., 2012. Toddlers' food preferences. The impact of novel food exposure, maternal preferences and food neophobia. *Appetite*, 59(3), pp.818–25.

Hunsberger, M., Lanfer, A., Reeske, A., Veidebaum, T., Russo, P., Hadjigeorgiou, C., Moreno, L.A., Molnar, D., De Henauw, S., Lissner, L. & Eiben, G., 2013. Infant feeding practices and prevalence of obesity in eight European countries - the IDEFICS study. *Public Health Nutrition*, 16(2), pp.219–227.

Ip, S., Chung, M., Raman, G., Chew, P., Magula, N., DeVine, D., Trikalinos, T. & Lau, J., 2007. Breastfeeding and maternal and infant health outcomes in developed countries. *Evidence report/technology assessment*, (153), pp.1–186.

Jacobi, C., Agras, W.S., Bryson, S. & Hammer, L.D., 2003. Behavioral validation, precursors, and concomitants of picky eating in childhood. *Journal of the American Academy of Child and Adolescent Psychiatry*, 42(1), pp.76–84.

Jonsdottir, O.H., Thorsdottir, I., Hibberd, P.L., Fewtrell, M.S., Wells, J.C., Palsson, G.I., Lucas, A., Gunnlaugsson, G. & Kleinman, R.E., 2012. Timing of the Introduction of Complementary Foods in Infancy: A Randomized Controlled Trial. *Pediatrics*, 130(6), pp.1038–1045.

Kelder, S.H., Perry, C.L., Klepp, K.I. & Lytle, L.L., 1994. Longitudinal tracking of adolescent smoking, physical activity, and food choice behaviors. *American journal of public health*, 84(7), pp.1121–6.

Kelly, Y., Panico, L., Bartley, M., Marmot, M., Nazroo, J. & Sacker, A., 2009. Why does birthweight vary among ethnic groups in the UK? Findings from the Millennium Cohort Study. *Journal of public health*, 31(1), pp.131–7.

King, C., 2009. An evidence based guide to weaning preterm infants. *Paediatrics and Child Health*, 19(9), pp.405–414.

Koletzko, B., Agostoni, C., Carlson, S.E., Clandinin, T., Hornstra, G., Neuringer, M., Uauy, R., Yamashiro, Y. & Willatts, P., 2001. Long chain polyunsaturated fatty acids (LC-PUFA) and perinatal development. *Acta paediatrica*, 90(4), pp.460–4.

Koletzko, B., Beyer, J., Brands, B., Demmelmair, H., Grote, V., Haile, G., Gruszfeld, D., Rzehak, P., Socha, P. & Weber, M., 2013. Early influences of nutrition on postnatal growth. *Nestlé Nutrition Institute workshop series*, 71, pp.11–27.

Koletzko, B., von Kries, R., Closa, R., Escribano, J., Scaglioni, S., Giovannini, M., Beyer, J., Demmelmair, H., Gruszfeld, D., Dobrzanska, A., Sengier, A., Langhendries, J.P., Rolland Cachera, M.F., Grote, V. & Kries, V., 2009. Lower protein in infant formula is associated with lower weight up to age 2 y: a randomized clinical trial. *Am J Clin Nutr*, 89(6), pp.1836–1845.

Kramer, M.S., Guo, T., Platt, R.W., Sevkovskaya, Z., Dzikovich, I., Collet, J.-P., Shapiro, S., Chalmers, B., Hodnett, E., Vanilovich, I., Mezen, I., Ducruet, T., Shishko, G. & Bogdanovich, N., 2003. Infant growth and health outcomes associated with 3 compared with 6 mo of exclusive breastfeeding. *The American journal of clinical nutrition*, 78(2), pp.291–5.

Kramer, M.S. & Kakuma, R., 2012. Optimal duration of exclusive breastfeeding. *The Cochrane database of systematic reviews*, 8(1), p.CD003517.

Kramer, M.S. & Kakuma, R., 2002. Optimal duration of exclusive breastfeeding. *The Cochrane database of systematic reviews*, 8(1), p.CD003517.

Krebs, N.F., 2000. Dietary zinc and iron sources, physical growth and cognitive development of breastfed infants. *J Nutr*, 130(Suppl), p.358S–360S.

Krebs, N.F., 2007. Meat as an early complementary food for infants: implications for macro- and micronutrient intakes. *Nestle Nutr Workshop Ser Pediatr Program*, 60, pp.221–233.

Krebs, N.F. & Hambidge, K.M., 2007. Complementary feeding: clinically relevant factors affecting timing and composition. *Am J Clin Nutr*, 85(Suppl), p.639S–645S.

Krebs, N.F., Reidinger, C.J., Robertson, A.D. & Hambidge, K.M., 1994. Growth and intakes of energy and zinc in infants fed human milk. *J Pediatr*, 124(1), pp.32–39.

Krebs, N.F., Sherlock, L.G., Westcott, J., Culbertson, D., Hambidge, K.M., Feazel, L.M., Robertson, C.E. & Frank, D.N., 2013. Effects of Different Complementary Feeding Regimens on Iron Status and Enteric Microbiota in Breastfed Infants. *The Journal of pediatrics*, 163(2), pp.416–423.

Krebs, N.F., Westcott, J.E., Butler, N., Robinson, C., Bell, M. & Hambidge, K.M., 2006. Meat as a first complementary food for breastfed infants: feasibility and impact on zinc intake and status. *J Pediatr Gastroenterol Nutr*, 42(2), pp.207–214.

Labbok, M. & Krasovec, K., 1990. Toward consistency in breastfeeding definitions. *Studies in family planning*, 21(4), pp.226–30.

Lande, B., Andersen, L.F., Veierød, M.B., Baerug, A., Johansson, L., Trygg, K.U. & Bjørneboe, G.A., 2004. Breast-feeding at 12 months of age and dietary habits among breast-fed and non-breast-fed infants. *Public health nutrition*, 7(4), pp.495–503.

Lange, C., Visalli, M., Jacob, S., Chabanet, C., Schlich, P. & Nicklaus, S., 2013. Maternal feeding practices during the first year and their impact on infants' acceptance of complementary food. *Food Quality and Preference*, 29(2), pp.89–98.

Lanigan, J. a, Wells, J.C.K., Lawson, M.S., Cole, T.J. & Lucas, a, 2004. Number of days needed to assess energy and nutrient intake in infants and young children between 6 months and 2 years of age. *European journal of clinical nutrition*, 58(5), pp.745–50.

Lanigan, J.A., Bishop, J., Kimber, A.C. & Morgan, J., 2001. Systematic review concerning the age of introduction of complementary foods to the healthy full-term infant. *Eur J Clin Nutr*, 55(5), pp.309–320.

De Lauzon-Guillain, B., Jones, L., Oliveira, A., Moschonis, G., Betoko, A., Lopes, C., Moreira, P., Manios, Y., Papadopoulos, N.G., Emmett, P. & Charles, M.A., 2013. The influence of early feeding practices on fruit and vegetable intake among preschool children in 4 European birth cohorts. *The American journal of clinical nutrition*, 98(3), pp.804–12.

De Lauzon-Guillain, B., Oliveira, A., Charles, M.A., Grammatikaki, E., Jones, L., Rigal, N., Lopes, C., Manios, Y., Moreira, P., Emmett, P. & Monnery-Patris, S., 2012. A review of methods to assess parental feeding practices and preschool children's eating behavior: the need for further development of tools. *Journal of the Academy of Nutrition and Dietetics*, 112(10), pp.1578–602, 1602 e1–8.

Leathwood, P. & Maier, A., 2005. Early influences on taste preferences. *Nestlé Nutrition workshop series. Paediatric programme*, 56, pp.127–38; discussion 138–41.

Lennox, A., Sommerville, J., Ong, K., Henderson, H. & Allen, R., 2013. *Diet and Nutrition Survey of Infants and Young Children, 2011*, Department of Health / Food Standards Agency.

Lozoff, B., Beard, J., Connor, J., Barbara, F., Georgieff, M. & Schallert, T., 2006. Long-lasting neural and behavioral effects of iron deficiency in infancy. *Nutr Rev*, 64(5), pp.S34–43.

Ludvigsson, J.F. & Fasano, A., 2012. Timing of introduction of gluten and celiac disease risk. *Annals of nutrition & metabolism*, 60(Suppl 2), pp.22–9.

Luke, B., 2005. Nutrition and multiple gestation. *Seminars in perinatology*, 29(5), pp.349–54.

Lynch, S.R., Skikne, B.S. & Cook, J.D., 1989. Food iron absorption in idiopathic hemochromatosis. *Blood*, 74(6), pp.2187–93.

Macdonald, P.D., Ross, S.R.M., Grant, L. & Young, D., 2003. Neonatal weight loss in breast and formula fed infants. *Archives of disease in childhood. Fetal and neonatal edition*, 88(6), pp.F472–6.

Maier, A., Chabanet, C., Schaal, B., Leathwood, P. & Issanchou, S., 2007. Food-related sensory experience from birth through weaning: contrasted patterns in two nearby European regions. *Appetite*, 49(2), pp.429–440.

Maier, A.S., Chabanet, C., Schaal, B., Leathwood, P.D. & Issanchou, S.N., 2008. Breastfeeding and experience with variety early in weaning increase infants' acceptance of new foods for up to two months. *Clin Nutr*, 27(6), pp.849–857.

Marriott, L.D., Robinson, S.M., Poole, J., Borland, S.E., Godfrey, K.M., Law, C.M. & Inskip, H.M., 2008. What do babies eat? Evaluation of a food frequency questionnaire to assess the diets of infants aged 6 months. *Public health nutrition*, 11(7), pp.751–6.

McAndrew, F., Thompson, J., Fellows, L., Large, A., Speed, M. & Renfrew, M.J., 2012a. *Infant Feeding Survey 2010*, Health and Social Care Information Centre.

McAndrew, F., Thompson, J., Fellows, L., Large, A., Speed, M. & Renfrew, M.J., 2012b. Infant Feeding Survey 2010 Chapter 8. Introduction of solid foods. In *Infant Feeding Survey 2010*. Health and Social Care Information Centre.

McDonald, S.J. & Middleton, P., 2008. Effect of timing of umbilical cord clamping of term infants on maternal and neonatal outcomes. *Cochrane Database Syst Rev*, (2), pp.1–67.

Mehta, K.C., Specker, B.L., Bartholmey, S., Giddens, J. & Ho, M.L., 1998. Trial on Timing of Introduction to Solids and Food Type on Infant Growth. *Pediatrics*, 102(3), pp.569–573.

Mennella, J.A., Lukasewycz, L.D., Castor, S.M. & Beauchamp, G.K., 2011. The timing and duration of a sensitive period in human flavor learning: a randomized trial. *Am J Clin Nutr*, 93(5), pp.1019–1024.

Mennella, J.A. & Ventura, A.K., 2011. Early feeding: setting the stage for healthy eating habits. *Nestle Nutr Workshop Ser Pediatr Program*, 68, pp.153–158.

Mihrshahi, S., Ampon, R., Webb, K., Almqvist, C., Kemp, A.S., Hector, D. & Marks, G.B., 2007. The association between infant feeding practices and subsequent atopy among children with a family history of asthma. *Clin Exp Allergy*, 37(5), pp.671–679.

Miles, J. & Shevlin, M., 2001. *Applying Regression and Correlation: A Guide for Students and Researchers*, Londbn: Sage Publications.

Moorcroft, K.E., Marshall, J.L. & McCormick, F.M., 2011. Association between timing of introducing solid foods and obesity in infancy and childhood: a systematic review. *Maternal & child nutrition*, 7(1), pp.3–26.

Morgan, J.B. & Dickerson, J.W.T. eds., 2003. *Nutrition in Early Life* 1st ed., Chichester: Wiley-Blackwell.

Morgan, J.B., Lucas, a & Fewtrell, M.S., 2004. Does weaning influence growth and health up to 18 months? *Archives of disease in childhood*, 89(8), pp.728–33.

Muñoz, M., Munoz, M., Villar, I. & Garcia-Erce, J.A., 2009. An update on iron physiology. *World Journal of Gastroenterology*, 15(37), p.4617.

Nadadur, S.S., Srirama, K. & Mudipalli, A., 2008. Iron transport & homeostasis mechanisms: their role in health & disease. *Indian J Med Res*, 128(4), pp.533–544.

Nicklaus, S., 2011. Children's acceptance of new foods at weaning. Role of practices of weaning and of food sensory properties. *Appetite*, 57(3), pp.812–5.

Nicklaus, S., 2009. Development of food variety in children. *Appetite*, 52(1), pp.253–5.

Nicklaus, S., Boggio, V. & Issanchou, S., 2005. Food choices at lunch during the third year of life: high selection of animal and starchy foods but avoidance of vegetables. *Acta paediatrica*, 94(7), pp.943–51.

Nielsen, S.B., Reilly, J.J., Fewtrell, M.S., Eaton, S., Grinham, J. & Wells, J.C., 2011. Adequacy of milk intake during exclusive breastfeeding: a longitudinal study. *Pediatrics*, 128(4), pp.e907–14.

Noble, S., Emmett, P. & ALSPAC Study Team, 2001. Food and nutrient intake in a cohort of 8-month-old infants in the south-west of England in 1993. *European journal of clinical nutrition*, 55(8), pp.698–707.

Nommsen-Rivers, L.A. & Dewey, K.G., 2009. Growth of breastfed infants. *Breastfeed Med*, 4(Suppl 1), pp.S45–9.

Northstone, K., Emmett, P. & Nethersole, F., 2001. The effect of age of introduction to lumpy solids on foods eaten and reported feeding difficulties at 6 and 15 months. *J Hum Nutr Diet*, 14(1), pp.43–54.

Ntouva A., Rogers I., MacAdam A., E.P., 2011. Weaning practices and iron status of exclusively breast fed infants. *Journal of Human Nutrition and Dietetics*, 24(3), pp.297–298.

Ntouva, A., Emmett, P., MacAdam, A., Rabe, H., Ranganathan, S., Williams, C. and Rogers, I.S., 2011. Very low dietary iron intakes but normal haemoglobin levels at 8 months among fully breastfed infants starting solids at around 6 months: Electronic response to: Fewtrell et al. Six months of exclusive breast feeding: how good is the evidence? *BMJ*, 342, p.c5955.

Nwaru, B.I., Erkkola, M., Ahonen, S., Kaila, M., Haapala, A.-M.M., Kronberg-Kippila, C., Salmelin, R., Veijola, R., Ilonen, J., Simell, O., Knip, M., Virtanen, S.M. & Kronberg-Kippilä, C., 2010. Age at the introduction of solid foods during the first year and allergic sensitization at age 5 years. *Pediatrics*, 125(1), pp.50–59.

Van Odijk, J., Hulthen, L., Ahlstedt, S. & Borres, M.P., 2004. Introduction of food during the infant's first year: a study with emphasis on introduction of gluten and of egg, fish and peanut in allergy-risk families. *Acta Paediatr*, 93(4), pp.464–470.

Ong, K.K., Emmett, P.M., Noble, S., Ness, A. & Dunger, D.B., 2006. Dietary energy intake at the age of 4 months predicts postnatal weight gain and childhood body mass index. *Pediatrics*, 117(3), pp.e503–8.

Ong, K.K. & Loos, R.J.F., 2006. Rapid infancy weight gain and subsequent obesity: systematic reviews and hopeful suggestions. *Acta paediatrica*, 95(8), pp.904–8.

Owen, C.G., Martin, R.M., Whincup, P.H., Davey-smith, G., Gillman, M.W. & Cook, D.G., 2005. The effect of breastfeeding on mean body mass index throughout life : a quantitative review of published and unpublished observational evidence 1 – 3 Part of the International. *American Journal of Clinical Nutrition*, 82(3), pp.1298–1307.

Papanikolaou, G. & Pantopoulos, K., 2005. Iron metabolism and toxicity. *Toxicol Appl Pharmacol*, 202(2), pp.199–211.

Park, J.S., Chang, J.Y., Hong, J., Ko, J.S., Seo, J.K., Shin, S. & Lee, E.H., 2012. Nutritional Zinc Status in Weaning Infants: Association with Iron Deficiency, Age, and Growth Profile. *Biological Trace Element Research*, 150(1-3), pp.91–102.

Patsourou, A., Konstantinides, T., Mantadakis, E., Tsalkidis, A., Zarras, C., Balaska, A., Simopoulos, K. & Chatzimichael, A., 2012. Growth of exclusively breastfed and self-weaned children of Greece aged 0-36 months. *Breastfeeding medicine : the official journal of the Academy of Breastfeeding Medicine*, 7(6), pp.521–5.

Paul, A.A., Black, A.E., Evans, J., Cole, T.J. & Whitehead, R.G., 1988. Breastmilk intake and growth in infants from two to ten months. *Journal of Human Nutrition and Dietetics*, 1(6), pp.437–450.

Perrine, C.G., Sharma, A.J., Jefferds, M.E., Serdula, M.K. & Scanlon, K.S., 2010. Adherence to vitamin D recommendations among US infants. *Pediatrics*, 125(4), pp.627–632.

Pliner, P. & Loewen, E.R., 1997. Temperament and food neophobia in children and their mothers. *Appetite*, 28(3), pp.239–54.

Pliner, P. & Salvy, S., 2006. Food neophobia in humans. In *Frontiers in Nutritional Science*. pp. 75–92.

Poslusna, K., Ruprich, J., de Vries, J.H.M., Jakubikova, M. & van't Veer, P., 2009. Misreporting of energy and micronutrient intake estimated by food records and 24 hour recalls, control and adjustment methods in practice. *The British journal of nutrition*, 101 (Suppl 2), pp.S73–85.

Rabe, H., Alvarez, R.F., Whitfield, T., Lawson, F. & Jungmann, H., 2010. Spectroscopic noninvasive measurement of hemoglobin compared with capillary and venous values in neonates. *Neonatology*, 98(1), pp.1–5.

Rabe, H., Stupp, N., Ozgün, M., Harms, E. & Jungmann, H., 2005. Measurement of transcutaneous hemoglobin concentration by noninvasive white-light spectroscopy in infants. *Pediatrics*, 116(4), pp.841–3.

Rao, R. & Georgieff, M.K., 2007. Iron in fetal and neonatal nutrition. *Seminars in fetal & neonatal medicine*, 12(1), pp.54–63.

Rao, R. & Georgieff, M.K., 2002. Perinatal aspects of iron metabolism. *Acta Paediatr Suppl*, 91(438), pp.124–129.

Rapley, G., 2005. Baby-led weaning. In V. Hall Moran & F. Dykes, eds. *Maternal and Infant Nutrition and Nurture: Controversies and Challenges*. Quay Books, pp. 275–299.

Reau, N.R., Senturia, Y.D., Lebailly, S.A. & Christoffel, K.K., 1996. Infant and toddler feeding patterns and problems: normative data and a new direction. Pediatric Practice Research Group. *J Dev Behav Pediatr*, 17(3), pp.149–153.

Redsell, S.A., Atkinson, P., Nathan, D., Siriwardena, A.N., Swift, J.A. & Glazebrook, C., 2010. Parents' beliefs about appropriate infant size, growth and feeding behaviour: implications for the prevention of childhood obesity. *BMC Public Health*, 10, pp.711–721.

Reilly, J.J., Ashworth, S. & Wells, J.C.K., 2007. Metabolisable energy consumption in the exclusively breast-fed infant aged 3–6 months from the developed world: a systematic review. *British Journal of Nutrition*, 94(01), p.56.

Reilly, J.J. & Wells, J.C.K., 2007. Duration of exclusive breast-feeding: introduction of complementary feeding may be necessary before 6 months of age. *British Journal of Nutrition*, 94(06), p.869.

Rendell, M., Anderson, E., Schlueter, W., Mailliard, J., Honigs, D. & Rosenthal, R., 2003. Determination of hemoglobin levels in the finger using near infrared spectroscopy. *Clinical and laboratory haematology*, 25(2), pp.93–7.

Rigal, N., Chabanet, C., Issanchou, S. & Monnery-Patris, S., 2012. Links between maternal feeding practices and children's eating difficulties. Validation of French tools. *Appetite*, 58(2), pp.629–637.

Robinson, S., Ntani, G., Simmonds, S., Syddall, H., Dennison, E., Sayer, A.A., Barker, D. & Cooper, C., 2013. Type of milk feeding in infancy and health behaviours in adult life: findings from the Hertfordshire Cohort Study. *The British journal of nutrition*, 109(6), pp.1114–22.

Robinson, S.M., Marriott, L.D., Crozier, S.R., Harvey, N.C., Gale, C.R., Inskip, H.M., Baird, J., Law, C.M., Godfrey, K.M. & Cooper, C., 2009. Variations in infant feeding practice are associated with body composition in childhood: a prospective cohort study. *J Clin Endocrinol Metab*, 94(8), pp.2799–2805.

Rogers, I.S. & Emmett, P.M., 2001. Fat content of the diet among preschool children in southwest Britain: II. relationship with growth, blood lipids, and iron status. *Pediatrics*, 108(3), p.E49.

Rowan, H. & Harris, C., 2012. Baby-led weaning and the family diet. A pilot study. *Appetite*, 58(3), pp.1046–9.

Ruel, M., 2003. Operationalizing dietary diversity: a review of measurement issues and research priorities. *The Journal of nutrition*, 133, p.3911S–3926S.

Sarkar, S., 2004. Nutritional aspects of breast milk. *Nutrition & Food Science*, 34(3), pp.151–155.

Savage, J.S., Fisher, J.O. & Birch, L.L., 2007. Parental influence on eating behavior: conception to adolescence. *The Journal of law, medicine & ethics : a journal of the American Society of Law, Medicine & Ethics*, 35(1), pp.22–34.

Schwartz, C., Chabanet, C., Laval, C., Issanchou, S. & Nicklaus, S., 2012. Breast-feeding duration: influence on taste acceptance over the first year of life. *Br J Nutr*, 109(6), pp.1–8.

Schwartz, C., Issanchou, S. & Nicklaus, S., 2009. Developmental changes in the acceptance of the five basic tastes in the first year of life. *The British journal of nutrition*, 102(9), pp.1375–85.

Schwartz, C., Scholtens, P. a M.J., Lalanne, A., Weenen, H. & Nicklaus, S., 2011. Development of healthy eating habits early in life. Review of recent evidence and selected guidelines. *Appetite*, 57(3), pp.796–807.

Scientific Advisory Committee on Nutrition, 2007a. *Application of WHO Growth Standards in the UK: Report prepared by the Joint SACN/RCPCH Expert Group on Growth Standards.*, London: The Stationery Office.

Scientific Advisory Committee on Nutrition, 2011a. *Dietary Reference Values for Energy*, London: The Stationery Office.

Scientific Advisory Committee on Nutrition, 2010. *Iron and Health*, London: The Stationery Office.

Scientific Advisory Committee on Nutrition, 2003. *Salt and Health*, London: The Stationery Office.

Scientific Advisory Committee on Nutrition, 2011b. *The influence of maternal, fetal and child nutrition on the development of chronic disease in later life*, London: The Stationery Office.

Scientific Advisory Committee on Nutrition, 2007b. *Update on Vitamin D*, London: The Stationery Office.

Scott, J.A., Chih, T.Y. & Oddy, W.H., 2012. Food variety at 2 years of age is related to duration of breastfeeding. *Nutrients*, 4(10), pp.1464–74.

Scott, J.A., Ng, S.Y. & Cobiac, L., 2012. The relationship between breastfeeding and weight status in a national sample of Australian children and adolescents. *BMC Public Health*, 12, pp.107–113.

Sherriff, A., Emond, A., Bell, J.C. & Golding, J., 2001. Should infants be screened for anaemia? A prospective study investigating the relation between haemoglobin at 8, 12, and 18 months and development at 18 months. *Arch Dis Child*, 84(6), pp.480–485.

Sherriff, A., Emond, A., Hawkins, N. & Golding, J., 1999. Haemoglobin and ferritin concentrations in children aged 12 and 18 months. *Archives of Disease in Childhood*, 80(2), pp.153–157.

Shim, J.E., Kim, J. & Mathai, R.A., 2011. Associations of infant feeding practices and picky eating behaviors of preschool children. *Journal of the American Dietetic Association*, 111(9), pp.1363–8.

Shreffler, W.G. & Radano, M., 2011. Food allergy and complementary feeding. *Nestlé Nutrition workshop series. Paediatric programme*, 68, pp.141–9; discussion 150–2.

Siddappa, A.M., Rao, R., Long, J.D., Widness, J.A. & Georgieff, M.K., 2007. The assessment of newborn iron stores at birth: a review of the literature and standards for ferritin concentrations. *Neonatology*, 92(2), pp.73–82.

Skinner, J.D., Carruth, B.R., Wendy, B. & Ziegler, P.J., 2002. Children's food preferences: a longitudinal analysis. *Journal of the American Dietetic Association*, 102(11), pp.1638–47.

Sloan, S., Gildea, a, Stewart, M., Sneddon, H. & Iwaniec, D., 2008. Early weaning is related to weight and rate of weight gain in infancy. *Child: care, health and development*, 34(1), pp.59–64.

Steiner, J.E., Glaser, D., Hawilo, M.E. & Berridge, K.C., 2001. Comparative expression of hedonic impact: affective reactions to taste by human infants and other primates. *Neuroscience and biobehavioral reviews*, 25(1), pp.53–74.

Stuff, J., Garza, C., Boutte, C., Fraley, J., Smith, E., Klein, E. & Nichols, B., 1986. Sources of variance in milk and caloric intakes in breast-fed infants: implications for lactation study design and interpretation. *Am J Clin Nutr*, 43(3), pp.361–366.

Sullivan, S.A. & Birch, L.L., 1994. Infant dietary experience and acceptance of solid foods. *Pediatrics*, 93(2), pp.271–277.

Tapiero, H., Gate, L. & Tew, K.D., 2001. Iron: deficiencies and requirements. *Biomed Pharmacother*, 55(6), pp.324–332.

The Soil Association, 2012. Organic market report 2012. Available at: http://www.soilassociation.org/LinkClick.aspx?fileticket=5QS24GNSZTA%3D&tabid=116.

Thompson, F.E. & Byers, T., 1994. Dietary assessment resource manual. *The Journal of nutrition*, 124(Suppl), p.2245S–2317S.

Townsend, E. & Pitchford, N.J., 2012. Baby knows best? The impact of weaning style on food preferences and body mass index in early childhood in a case-controlled sample. *BMJ open*, 2(1), p.e000298.

Ventura, A.K. & Birch, L.L., 2008. Does parenting affect children's eating and weight status? *The international journal of behavioral nutrition and physical activity*, 5(15), p.15.

Ventura, A.K. & Worobey, J., 2013. Early influences on the development of food preferences. *Current biology*, 23(9), pp.R401–8.

Wardle, J. & Cooke, L., 2008. Genetic and environmental determinants of children's food preferences. *The British journal of nutrition*, 99 Suppl 1, pp.S15–21.

Weaver, L., 2012. A short history of infant feeding and growth. *Early human development*, 88 Suppl 1, pp.S57–9.

Wells, J. & Jonsdottir, O., 2012. Randomized controlled trial of 4 compared with 6 mo of exclusive breastfeeding in Iceland: differences in breast-milk intake by stable-isotope probe. *The American journal of Clinical Nutrition*, 96(1), pp.73–9.

White, R. & Allen, R., 2013. Annexe D . Composition of breast milk review. In *Diet and Nutrition Survey of Infants and Young Children, 2011*. Department of Health / Food Standards Agency.

Willett, W.C., Howe, G.R. & Kushi, L.H., 1997. Adjustment for total energy intake in epidemiologic studies. *The American journal of clinical nutrition*, 65(Suppl), p.1220S–1228S; discussion 1229S–1231S.

Williams, C. & Pickin, M., 2010. *Normal,Supported and Rewarding: A Breastfeeding strategy for Brighton & Hove 2009/10-2011/12*, NHS Brighton and Hove.

Witt, M. & Reutter, K., 1996. Embryonic and early fetal development of human taste buds: a transmission electron microscopical study. *The Anatomical record*, 246(4), pp.507–23.

World Health Organisation, 1995. An evaluation of infant growth: the use and interpretation of anthropometry in infants. WHO Working Group on Infant Growth. *Bull World Health Organ*, 73(2), pp.165–174.

World Health Organisation, 2002. *Global strategy for infant and young child feeding*, Geneva: WHO.

World Health Organization, 2001. *Iron deficiency anaemia: Assessment, Prevention and Control*, Geneva: WHO.

World Health Organization, 2002. *The World Health Report 2002: Reducing risks, promoting healthy life.*, Geneva: WHO.

World Health Organization, 2008. *Worldwide prevalence of anaemia 1993-2005*, Geneva: WHO.

World Health Organization & Centers for Disease Control and Prevention, 2007. *Assessing the iron status of populations : including literature reviews : report of a Joint World Health Organization/Centers for Disease Control and Prevention Technical*

Consultation on the Assessment of Iron Status at the Population Level. 2nd ed., Geneva: WHO.

Wright, C.M., Parkinson, K.N. & Drewett, R.F., 2004. Why are babies weaned early? Data from a prospective population based cohort study. *Archives of disease in childhood*, 89(9), pp.813–6.

Ziegler, E.E. & Nelson, S.E., 2010. Growth charts compared. *Nestlé Nutrition workshop series. Paediatric programme*, 65, pp.197–210; discussion 210–2.

Zimmermann, M.B., 2008. Methods to assess iron and iodine status. *The British journal of nutrition*, 99 Suppl 3, pp.S2–9.

Zimmermann, M.B. & Hurrell, R.F., 2007. Nutritional iron deficiency. *Lancet*, 370(9586), pp.511–520.

Zutavern, A., Brockow, I., Schaaf, B., von Berg, A., Diez, U., Borte, M., Kraemer, U., Herbarth, O., Behrendt, H., Wichmann, H.-E.E. & Heinrich, J., 2008. Timing of solid food introduction in relation to eczema, asthma, allergic rhinitis, and food and inhalant sensitization at the age of 6 years: results from the prospective birth cohort study LISA. *Pediatrics*, 121(1), pp.e44–52.

Appendix 1

Supporting Documents for The Brighton's Breast-fed Babies Study and the Haemospect® Study

8 Appendices

NHS

National Research Ethics Service

West Kent Research Ethics Committee

South East Coast Strategic Health Authority
Preston Hall
Aylesford
Kent
ME20 7NJ

Telephone: 01622 713012
Facsimile: 01622 885966

30 September 2009

Ms Antiopi Ntouva
Pharmacy and Biomolecular Sciences
University of Brighton
Cockcroft Building
Lewes Road
Brighton
Sussex
BN2 4GJ

Dear Ms Ntouva

Study Title:	**The association between the age of introduction of solid foods to breastfed infants, iron status and diet composition at ages 8 and 18 months: The Brighton's Breastfed Babies Study.**
REC reference number:	**09/H1101/55**
Protocol number:	**2**

Thank you for your letter of 10th September 2009, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information was considered in correspondence by a sub-committee of the REC. A list of the sub-committee members is attached.

Confirmation of ethical opinion

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

Ethical review of research sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

<u>Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.</u>

[redacted]

For NHS research sites only, management permission for research ("R&D approval") should be obtained from the relevant care organisation(s) in accordance with NHS research governance arrangements. Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk. *Where the only involvement of the NHS organisation is as a Participant Identification Centre, management permission for research is not required but the R&D office should be notified of the study. Guidance should be sought from the R&D office where necessary.*

Sponsors are not required to notify the Committee of approvals from host organisations.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

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

Document	*Version*	*Date*
Volunteer postcard	2	20 May 2009
Clinical Support Email fromPaediatrician		03 June 2009
Supervisor CV - Imogen Rogers		14 March 2009
GP/Consultant Information Sheets	2	20 May 2009
Questionnaire: 18 month questionnaire	2	20 May 2009
Questionnaire: 8 month questionnaire	2	20 May 2009
Questionnaire: Background Questionnaire	2	20 May 2009
Compensation Arrangements		04 June 2008
Letter from Sponsor		23 June 2009
Covering Letter		23 June 2009
Investigator CV	Antiopi Ntouva	11 June 2009
REC application		23 June 2009
Unfavourable Opinion Letter from Brighton East REC		15 April 2009
Parents' anaemia letter	2	20 May 2009
Service managers letter	2	20 May 2009
Portion size guide	2	20 May 2009
Data recording sheet	2	20 May 2009
Breastfeeding support groups		
Baby clinics		
GP surgeries		
Heel prick assessment tool		
Peer Review		23 June 2009
Participant Information Sheet	3	01 September 2009
Response to Request for Further Information		
Protocol	3	01 September 2009
Participant Consent Form: Questionnaires & Food Diaries	3	01 September 2009
Advert for local press & poster	3	01 September 2009
Questionnaire: 8 month food diary	3	01 September 2009
8 month crib sheet	1	01 September 2009
Questionnaire: 18 month food diary	3	01 September 2009

[redacted]

18 month crib sheet	1	01 September 2009
Participant Consent Form: Blood sampling	3	01 September 2009
Covering letter confirming that the blood tests are going to be carried out at the Royal Sussex County Hospital		10 September 2009

Statement of compliance

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

After ethical review

Now that you have completed the application process please visit the National Research Ethics Service website > After Review

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

We would also like to inform you that we consult regularly with stakeholders to improve our service. If you would like to join our Reference Group please email referencegroup@nres.npsa.nhs.uk.

09/H1101/55	**Please quote this number on all correspondence**

Yours sincerely

Professor V M Mathew
Acting Chair

Email: s.boland@nhs.net

Enclosures:	*List of names and professions of members who were present at the meeting and those who submitted written comments*
	"After ethical review – guidance for researchers"
Copy to:	*Miss Hilary Ougham, Pharmacy and Biololecular Sciences* *Ms Lorraine Southby, R&D office for South Downs NHS Trust*

[redacted]

Sussex NHS Research Consortium

Research Consortium Office
Worthing Hospital
Lyndhurst Road
Worthing
West Sussex
BN11 2DH

Tel: 01903 285027
Fax: 01903 209884
www.sxrc.nhs.uk

Ms Antiopi Ntouva
MPhil/PhD Student
University of Brighton
Pharmacy and Biomolecular Sciences
Cockcroft Building
Lewes Road
Brighton
BN2 4GJ

26/10/2009

Dear Ms Ntouva,

Our ID: 1269/NOCI/2009
TITLE: The association between the age of introduction of solid foods to breastfed infants, iron status and diet composition at ages 8 and 18 months: The Brighton's Breastfed Babies Study.

Thank you for your application to the Sussex NHS Research Consortium for research governance approval of the above named study.

I am pleased to inform you that the study has been approved, and so may proceed. This approval is valid in the following Organisations:

- **NHS Brighton & Hove:**
    1. **7 Dials Medical centre**
    2. **Dr Nalletamby and Partners, 30-36 Oxford Street**
    3. **The Surgery, Hove Park Villas**
    4. **Portslade Health Centre Medical Practice**
    5. **Saltdean & Brighton Medical Practice**
    6. **Wish Park Surgery**
- **South Downs Health NHS Trust:**
    1. **Hollingdean Children's Centre (Postnatal Group and Postnatal Support Group)**

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

- IRAS Form (submission code: 23878/46261/1/663)
- SSI Form (submission code: 23878/66365/6/525/33244/153051)
- Study Protocol (version 3, dated 01/09/2009)
- Advert for local press (version 3, dated 01/09/2009)
- Letter to Service Managers (version 2, dated 20/05/2009)
- Child Health Clinics (no version control, undated, received 22/10/2009)
- Breastfeeding Welcome (no version control, undated, received 22/10/2009)
- Food portion sizes guide (no version control, undated, received 22/10/2009)
- Heel prick competency (no version control, undated, received 22/10/2009)
- Participant Information Sheet (version 3, dated 01/09/2009)
- Parental Consent Form: Questionnaires and Food Diaries (version 3, dated 01/09/2009)
- Parental Consent Form: Blood sampling (version 3, dated 01/09/2009)
- Background information questionnaire (version 2, dated 20/05/2009)
- 8 Month Crib Sheet (version 1, dated 01/09/2009)

- 8 Month Food Diary (version 3, dated 01/09/2009)
- 8 Month General Questionnaire (version 2, dated 20/05/2009)
- 18 Month Crib Sheet (version 1, dated 01/09/2009)
- 18 Month Food Diary (version 3, dated 01/09/2009)
- 18 Month General Questionnaire (version 2, dated 20/05/2009)
- Data Recording Sheet (version 2, dated 20/05/2009)
- Parent's anaemia letter (version 2, dated 20/05/2009)
- GP anaemia letter (version 2, dated 20/05/2009)

Please note this additional condition of approval:

- **You must send us authorisation letters for additional GP surgeries and Clinics recruited within NHS Brighton & Hove and South Downs Health NHS Trust**

Your research governance approval is valid providing you comply with the conditions set out below:
1. You commence your research within one year of the date of this letter. If you do not begin your work within this time, you will be required to resubmit your application.
2. You notify the Consortium Office should you deviate or make changes to the approved documents.
3. You alert the Consortium Office by contacting me, if significant developments occur as the study progresses, whether in relation to the safety of individuals or to scientific direction.
4. You complete and return the standard annual self-report study monitoring form when requested to do so at the end of each financial year. Failure to do this will result in the suspension of research governance approval.
5. You comply fully with the Department of Health Research Governance Framework, and in particular that you ensure that you are aware of and fully discharge your responsibilities in respect to Data Protection, Health and Safety, financial probity, ethics and scientific quality. You should refer in particular to Sections 3.5 and 3.6 of the Research Governance Framework.
6. You ensure that all information regarding patients or staff remains secure and strictly confidential at all times. You ensure that you understand and comply with the requirements of the NHS Confidentiality Code of Practice, Data Protection Act and Human Rights Act. Unauthorised disclosure of information is an offence and such disclosures may lead to prosecution.

Good luck with your work.

Yours sincerely,

Miss Emma Peskett
Research Governance Officer

Email: emma.peskett@wsht.nhs.uk
Tel: 01903 285222 Ext 4781
Fax: 01903 209884

cc: Lorraine Southby, Acting R&D Manager, South Downs Health NHS Trust
Natasha Darby, Information and Research Manager, NHS Brighton

Weight measurements

Weight measurements will be taken by at 6, 8 and 18 months at the participants' homes.

Infants and toddlers will be weighed nude, without a nappy.

Toddlers who are unsettled or distracted will be held by a parent and both weighed together. The parent's weight can then be taken separately and subtracted from the total weight. Alternatively the parent can sit on the scales, which are zeroed. The parent, while still on the scales, can holds the child and both can be weighed together, so the scales show the weight of the child only

Measurements will be made in kilograms (kg) and grams (g).

The scale should be zeroed and cleaned. A paper sheet can be put on the surface.

The mother will place the infant on the scales.

The infant should stay still for a few seconds until the measurement can be taken.

The measurement will be taken and recorded.

The scales will be cleaned as per instruction manual.

Length measurements

Length measurements will be taken by at 6, 8 and 18 months at the participants' homes.

Up until a toddler's second birthday, length is measured lying down .

The length of the toddler should be measured without any clothing, including the nappy, which can distort the hips and shorten the length measurement.

The mat should be clean. A paper sheet can be put on the surface.

The mother will place the infant on the mat.

Two people are needed to obtain an accurate length measurement. The toddler should lie on his or her back with one person (ideally the parent) holding the head against the headboard with both hands. The corner of the toddler's eye should be in a vertical line with the middle of the ear (known as the Frankfort Plane). The researcher should gently flatten the knees and flex the ankles of the toddler to 90 degrees and bring the footboard up to the flat soles of the flexed feet.

The length measurement is then read off the scale, to the nearest 1/2cm.

A rollameter or a Holtain lying stadiometer is usually used. Most equipment is either self calibrating or comes with a one-metre standard measure with which to calibrate before use.

It is important to correct for prematurity in the first two years of life. A toddler under two years of age, born at 31 weeks gestation, for instance, was born nine weeks early (40 – 31 = 9 weeks). This child's corrected age will always be nine weeks less than his or her actual age since birth. When calculating the corrected age, subtract nine weeks from the actual age since birth date. For example, when the child is one year and 26 weeks old the corrected age will be one year and 17 weeks (26 – 9 = 17 weeks).

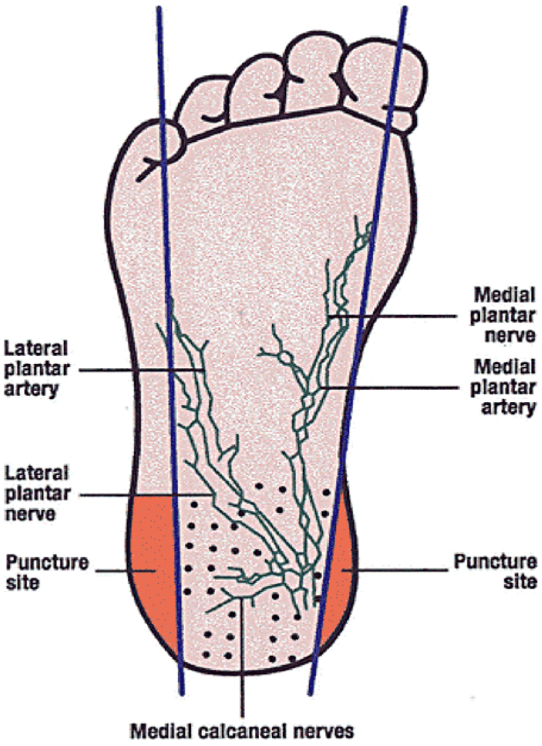
Heel prick measurements

Heel prick blood samples will be taken at 8 and 18 months at the participants' homes
The lancing device used will be Tenderfoot newborn (1mmx 2.5mm) blue/pink
Make sure baby is in a warm room, wearing socks to keep heel warm.
Ensure the infant is in a secure position, ideally cuddled by the parent
Wash hands and put powder free gloves
Ask parent to remove socks

Clean the area with antiseptic swab and wait until it dries.
Apply a thin layer of paraffin

Remove the device from its packaging

Remove the safety clip



Select a safe incision site. The heel prick should be performed on the planter surface of the heel beyond the lateral and medial limits of the calcaneous.

“Marked by a line extending posteriorly from a point between the 4th and 5th toes and running parallel to the lateral aspect of the heel, and a line extending posteriorly from the middle of the great toe running parallel to the medial aspect of the heel “

Before activating the device, ensure that the heel is warm and the device is placed firmly against the skin. The centre point of the device should be vertically aligned with the incision site and both ends of it should make light contact with the skin.

Press the trigger and remove device.

Wipe the first drop of blood with a sterile gauze

15.Transfer the blood drops with Plastic Pasteur pipettes to the suitable containers (lithium heparin for ferritin and EDTA for haemoglobin) without making direct wound contact with the pipette.

16. Gently massage the heel for increasing blood flow and wait for capillary refill. Do not squeeze.

17. Apply gentle pressure with gauze to stop bleeding. Place a plaster and dispose of sharps and other material.

18. Make sure samples are properly labelled. Put samples in transfer box and deliver to the Royal Sussex Hospital as soon as possible.

Brighton's Breast-fed Babies Study
University of Brighton
Cockcroft Building
Brighton
BN2 4GJ

Tel:

Permission to use questionnaire and food diary information

All questionnaires and food diaries will be identified by a study number, and will have no names attached. Results will be used for statistical purposes only and not linked to named mothers or children. Participation in the study is voluntary, and you may withdraw from the study at any time.

PARENTAL CONSENT

The purposes and possible risks in taking part in this study have been explained to me.

I agree to the information in the questionnaires and food diaries being used for research purposes.

I am his/her parent (or guardian).

Signed:…………………………………………………….

Date:………………………………………

Name (PLEASE PRINT)………………………………………………………..
(Parent/Guardian)

Name (PLEASE PRINT)……………………………………………………. (Study child)

The Brighton's Breast-fed Babies Study is covered by the University of Brighton's Insurance scheme

University of Brighton

Brighton's Breast-fed Babies Study

Cockcroft Building
Lewes Road
Brighton
BN2 4G

Tel:

Parental consent for blood sampling

Is your child taking any medication containing sulphonamides (such as Septrin or Cotrimoxazole)? Y/N

Is your child anaemic? Y/N

Does your child suffer from any clotting or bleeding disorders? Y/N

Has your child had an infection in the last 4 weeks? Y/N

Signed……………………………………………

Date..

Permission to use blood samples

If sample 1 has been taken, you will be informed if analysis of the blood sample shows that your child is anaemic*. After that, the link between the blood sample results and your child's name will be removed. The blood sample will be destroyed after analysis. Results will be used for statistical purposes only and not linked to named children.

PARENTAL CONSENT

The purposes and possible risks in having blood taken have been explained to me. (Please tick)

☐ I agree to my son/daughter having blood samples taken for analyses for the "Brighton's Breast-fed Babies" study:

1. Heparin sample for biochemical tests
2. EDTA sample for biochemical tests

(Heparin and EDTA are substances that prevent blood from clotting so that the results from the blood test are valid)

☐ I agree for the use of non-invasive white-light spectroscopy for haemoglobin measurements.

I am his/her parent (or guardian).

Signed: …………………………………………………….

Date: ....

Name (PLEASE PRINT)……………………………………………………..
(Parent/Guardian)

Name (PLEASE PRINT)……………………………………………………. (Study child)

***** Haemoglobin below 110 g/l or Ferritin below 12 micrograms/l**.**

The Brighton's Breast-fed Babies Study is covered by the University of Brighton's Insurance scheme

Background questionnaire

We would be very grateful if you could answer the following questions, to give us some background information on your pregnancy, your baby and your family.
If you do not wish to answer any of the questions please leave blank.

1. Your date of birth Day ☐☐ Month ☐☐ Year ☐☐☐☐

2. Baby's date of birth Day ☐☐ Month ☐☐ Year ☐☐☐☐

3. Baby's due date Day ☐☐ Month ☐☐ Year ☐☐☐☐

4. Baby's birth weight ☐☐☐☐ g OR ☐☐lb ☐☐oz

5. Before the birth of your study child, how many pregnancies have you had which resulted in either a live birth or a still birth? ☐☐

6. Are you currently living with a husband or partner?

Yes ☐ No ☐ (if no please go to question 8).

7. Partner's age ☐☐ Years

8. Including your study child, how many children do you have living with you in total? ☐☐

9. What educational qualifications do you and your partner have? Please tick all that apply

	You		Your partner	
GCSEs / O-levels	☐		☐	
A-levels	☐		☐	
Degree	☐		☐	
Postgraduate qualification	☐		☐	
No qualifications	☐		☐	
Other (please specify)	☐	________________	☐	________________

10. Your present job or last main job

Actual job, occupation, trade or profession

………………………………………………………………………………………..

Hours worked per week:………………………

11. Your partner – present job or last main job

Actual job, occupation, trade or profession

………………………………………………………………………………………..

Hours worked per week:………………………

12. Which ethnic group best describes you?

a. White	☐	f. Black African	☐
b. Indian	☐	g. Chinese	☐
c. Pakistani	☐	h. Mixed race	☐
d. Bangladeshi	☐	i. Other	☐
e. Black Caribbean	☐	(please describe)______________	

13. Which ethnic group best describes your partner?

a. White	☐	f. Black African	☐
b. Indian	☐	g. Chinese	☐
c. Pakistani	☐	h. Mixed race	☐
d. Bangladeshi	☐	i. Other	☐
e. Black Caribbean	☐	(please describe)_______________	

14. Is your home:

Being bought/mortgaged ☐
Owned – with no mortgage to pay ☐
Rented from council ☐
Rented from private landlord ☐
Other (please describe) ☐
....

15. Do you live in your own home or do you live with your parents or others?

Live in own home ☐
Live with parents in their home ☐
Other situation (please describe) ☐
……………………………………………………………………………………………

16.a) Have you ever been a smoker?
Yes, I am currently a smoker ☐
Yes, I used to smoke but have given up ☐
No, I have never smoked ☐

b) If you are currently smoking, how many cigarettes/times a day are you smoking at the moment

30+ ☐ 25-29 ☐ 20-24 ☐ 15-19 ☐

10-14 ☐ 5-9 ☐ 1-4 ☐ <1 ☐

17) Were you prescribed any iron supplements during your pregnancy?

Yes ☐

No ☐

If Yes, please describe ....

GENERAL QUESTIONS about your baby's food and drink
We hope that by answering these questions you won't have to keep repeating these details on the daily food questionnaire

1. What type of milk does your baby have mostly? We understand that you may be using several different sorts of milk. Please add more detail if you would like to

a. Breast milk: ☐

b. Formula milk: infant ☐ follow-on ☐ soya ☐ other formula ☐

If formula, which brand do you use?
____

c. Cows' milk: whole milk ☐ semi skimmed milk ☐ skimmed milk ☐

d. Soya milk (not formula) ☐

e. Other milk ☐

Please describe

____
We will assume that you have used the same milk throughout the day unless you tell us otherwise

2. If using formula please describe how you make the feed.

Are scoops usually? Rounded ☐ flat ☐ heaped ☐ don't use scoops ☐

3. Do you add anything else to the milk? a. Yes ☐ No ☐

If yes, please describe____

b. Number of times a day ☐☐

4. What sort of water does your baby usually drink? Tap water ☐ Filtered water ☐ Bottled water ☐

If bottled water which type and brand? ____

5. How many times a day does your baby drink from a

Bottle with a teat ☐☐ Feeding cup with lid ☐☐ Cup without a lid ☐☐

6. To help us decide on the correct amount of fluids for drinks, please fill your baby's **cup** with water to the usual level, then empty the water into a measuring jug and record here.
Fluid Ounces ☐☐ or Millilitres ☐☐☐ Doesn't use ☐
(fl. oz.) (ml) a cup

7. What age was your baby when you stopped breast-feeding? ☐☐ weeks or ☐ months

☐ still breast feeding

8. At what age did you introduce smooth, pureed foods to your baby's diet e.g. baby rice? ☐☐weeks or ☐ months

☐ haven't introduced yet

If you have not yet introduced solid foods, you do not need to answer the rest of the questions on this questionnaire

9. If you started to give your baby solid foods before he was six months old, why did you do so?

☐ Not applicable, waited until 6m ☐ Felt baby was ready for solid food ☐ Baby no longer satisfied by breast milk

☐ Advice from partner ☐ Advice from friends/relatives ☐ Advice from health professional

☐ Worried about baby's weight or growth ☐ Other reason, please describe

____

____

10. What was the first type of solid food that you gave to your baby? (please describe)

____

11. At what age did you start giving foods with a lumpier texture e.g. noodles? ☐☐ weeks or ☐ months

☐ haven't introduced yet

12. At what age did your baby start eating finger foods e.g. toast? ☐☐ weeks or ☐ months

☐ haven't introduced yet

13. Does your baby have definite likes and dislikes as far as food is concerned?

☐ no, will eat almost anything ☐ yes, quite choosy ☐ yes, very choos

14. Since starting solid foods, do you feel that you have had difficulty feeding your baby

☐ Yes, great difficulty ☐ Yes, some difficulty ☐ Yes, occasional difficulty

☐ No, no difficulty

15. Since starting solid foods, has your baby at any time in the past 2 months:

	Yes, worried me greatly	Yes, worried me a bit	Yes, but did not worry me	No, did not happen
a) Not eaten sufficient amount of food	☐	☐	☐	☐
b) Refused to eat the right food	☐	☐	☐	☐
c) Been choosy with food	☐	☐	☐	☐
d) Over-eaten	☐	☐	☐	☐
e) Been difficult to get into an eating routine	☐	☐	☐	☐
f) Any other problems with feeding (please describe)	☐	☐	☐	☐

____

____

16. What type of margarine or butter do you usually use for your baby? (If not used at all please go to question 17).
Please give the full name and brand from the packet of the type used most often.

---

17. What type of bread does your baby eat most often?
We will assume that you used the same bread and butter or margarine throughout the day.
Please indicate on the food diary page if there are any times that a different bread, butter or margarine is used.

White ☐ brown ☐ whole meal ☐

Granary ☐ high fibre white ☐ other ☐

Please give full description ____

18. What size loaf do you usually use?

Small loaf ☐ medium loaf ☐ large loaf ☐

How is your bread usually sliced?

Sliced (thin) ☐ sliced (medium) ☐ sliced (thick) ☐ unsliced ☐

19. Do you avoid giving your baby any of the following foods? Yes ☐ No ☐

Please tick all that apply

a) poultry	b) fish	c) beef	d) other red meat	e) eggs

f) cheese	g) milk	h) butter	i) nuts	j) wheat/gluten
k) other (please describe)				

20. Is your baby on any kind of special diet? Yes ☐ No ☐

If yes, please describe____

21. In your opinion, does your child eat differently than other children of the same age? Yes ☐ No ☐

If yes, please describe____

The Brighton's Breast-fed Babies Study

Food Diary

Date

____

Day of the week

____

Study number

How to fill in the diary

Now that your baby is eight months old we would like to look at his or her diet. Thank you very much for helping us by filling in these food diaries.

Please could you record everything that your baby has by mouth for 3 days this week. There are 3 diary booklets, one for each day, with different pages for you to record what your baby eats and drinks. Start each diary booklet when you get up in the morning and fill in everything your baby eats and drinks for a 24-hour period until the same time the next day. Please choose one weekend day and two weekdays. They need not be one after the other. If any day is likely to be very difficult or unusual choose another day. It is very important that you do not change what your baby normally eats and drinks just because you are keeping this record.

Try to fill in the pages as you go through the day as this is much easier and more accurate than trying to remember at the end of the day. If someone else looks after your baby for some of the time it would be most helpful if they could fill in the food given in the parts of the day when your baby is with them.

When recording the food given please include the brand name (if known), portion size (using feeding jar size, cup or spoon size, weights from labels), any additions to the food (fats, oils, sugar/sweeteners, sauces, salt, pepper etc.) and cooking methods (fried, grilled, micro-waved, roasted). We have given you a booklet “How to describe your baby’s food and drink” which shows you the sort of detail we need, and there are also some examples given in the diary.

Recipes/homemade dishes

If your baby has eaten any **homemade dishes** e.g. chicken casserole, please record the name of the recipe, ingredients with amounts (including water or other fluids) for the whole recipe, the number of people the recipe is for, and the cooking method. Record how much of the whole recipe your toddler ate.

Take-aways and eating out

If your baby has eaten **take-aways** or **made up dishes not prepared at home** such as at a restaurant or a friend’s house, please record as much detail about the ingredients as you can e.g. spaghetti with mince, onion and tomato sauce.

Labels/wrappers

These are an important source of information for us. It helps us a great deal if you enclose, in the plastic bag provided, labels from all ready meals, labels from foods of lesser known brands and also from any supplements your baby takes.

Our researcher will go through the food diaries with you at her next visit. If you can’t manage all 3 days then one or two complete diaries are still very useful to us.

Please complete the inside pages as you go through the day and this page at the end of the day.
Please put a ☑ in the box beside your answer

1. Did anyone else look after your baby during any mealtimes today?

No ☐ Yes ☐ If yes please indicate which meals.

Breakfast ☐ Midday meal ☐ Evening meal ☐

Who looked after your baby at these mealtimes? Partner ☐ Relation ☐

Friend ☐ Nanny or minder ☐ Nursery ☐ Playgroup ☐ Other ☐ ____________________

2. As far as you know was all the food and drink taken by your baby during this 24 hours recorded?

Yes ☐ Not sure ☐ No ☐

3. Was the food and drink for this 24 hours fairly typical of your baby?

Yes ☐ No ☐

If no, please describe how it differed from normal and if your baby was unwell during this 24 hours.

____

____

Has anyone else looked after your baby today? Yes ☐ No ☐

Please record any parts of this 24-hour period when someone else looked after your baby.		
Start Time (e.g. 9.30am)	Return Time (e.g. 12.00 noon)	Time elapsed

Record here any FOODS your baby EATS in this 24 hours.

See following sections to record milk-feeds and other drinks.
Please start each food on a new line

Each day is divided into time intervals, from before breakfast to the evening meal and through the night. Please find the appropriate time interval and write in the time when your baby eats something. Don't forget to include snacks like sweets, chocolate, biscuits, crisps, ice cream or fruit and spreads e.g. margarine .Also include any sugar added to cereals or drinks.

YOUR BABY'S FOOD

Time interval	**Time**	**Description of food and amount eaten** **Please give as much detail as possible including the raw ingredients and cooking method of all homemade foods. Also please give us a full description, including the brand and flavour, of other meals given with any liquid, sauces or foods you added.**
Before Breakfast		
Breakfast		
Mid-morning		

Please tell us if you have used a different type of bread or margarine/butter from that recorded on the GENERAL FOOD AND DRINK QUESTIONS *questionnaire.*

Foods continued
Record here all the FOODS your baby EATS, from lunchtime and through the rest of the day and evening, along with how much he or she ate.
See following sections to record milk-feeds and other drinks.

Time interval	**Time**	**Description of food and amount eaten** **Please give as much detail as possible including the raw ingredients and cooking method of all homemade foods. Also please give us a full description, including the brand and flavour, of other meals given with any liquid, sauces or foods you added.**
Lunch		
Tea Between lunch and evening meal		
Evening meal		
Later evening and through the night		

Please tell us if you have used a different type of bread or margarine/butter from that recorded on the GENERAL FOOD AND DRINK QUESTIONS *questionnaire.*

If your baby has a MILK-FEED at any time during this 24 hours, please fill in this section.

Time	**Bottle/cup feed of milk** How much did your baby drink? Fluid ounces (fl. Oz.) or Millilitres (ml)	**Breast feed** Number of minutes the feed lasted

Whatever other DRINKS your baby has, including water, in this 24 hours can be recorded here.
Please find the appropriate time slot and then record the time your baby has a drink.
Note the type of fruit juice, squash or fizzy drink etc. Please also include and vitamins or medicines on this sheet.

Time interval	**Time**	**Full description and/or brand of drink. Please remember any vitamins or medicines.**	**Did you dilute with water? yes/no**	**How much concentrate did you use?**	**Did you add sugar? Number of teaspoons?**	**How much did your baby drink?**
Before breakfast						
Breakfast						
Mid-morning						
Lunch						
Tea – between lunch and evening meal						
Evening meal						
Later evening – and through the night						

Please write any notes, comments or questions here.

GENERAL QUESTIONS about your baby's food and drink (18 months)
We hope that by answering these questions you won't have to keep repeating these details on the daily food questionnaire

1. What type of margarine or butter do you usually use for your baby?
(If not used at all please go to question 2).
Please give the full name and brand from the packet of the type used most often.

---

2. What type of bread does your baby eat most often?
We will assume that you used the same bread and butter or margarine throughout the day.
Please indicate on the food diary page if there are any times that a different bread, butter or margarine is used.

White ☐ brown ☐ whole meal ☐

Granary ☐ high fibre white ☐ other ☐

Please give full description ______________________________

3. What size loaf do you usually use?

Small loaf ☐ medium loaf ☐ large loaf ☐

How is your bread sliced?

Sliced (thin) ☐ sliced (medium) ☐ sliced (thick) ☐ unsliced ☐

4. Do you avoid giving your baby any of the following foods? Yes ☐ No ☐

Please tick all that apply

a) poultry	b) fish	c) beef	d) other red meat	e) eggs
f) cheese	g) milk	h) butter	i) nuts	j) wheat/gluten
k) sugar	l) other (please describe)			

5. Is your baby on any kind of special diet?
   Yes ☐ No ☐

If yes, please describe____

6. What type of milk does your baby have mostly. We understand that you may be using several different sorts of milk. Please add more detail if you would like

a. Breast milk: ☐

b. Formula milk: infant ☐ follow-on ☐ soya ☐ other formula ☐

If formula, which brand do you use?

____

c. Cows' milk: whole milk ☐ semi skimmed milk ☐ skimmed milk ☐

d. Soya milk (not formula) ☐

e. Other milk ☐

Please describe

We will assume that you have used the same milk throughout the day unless you tell us otherwise

7. If using formula please describe how you make the feed.

Are scoops usually? Rounded ☐ flat ☐ heaped ☐ don't use scoops ☐

8. Do you add anything else to the milk? a. Yes ☐ No ☐

If yes please describe____

b. Number of times a day ☐☐

9. What sort of water does your baby usually drink? Tap water ☐ Filtered water ☐ Bottled water ☐

If bottled water which type and brand? _______________________________________

10. How many times a day does your baby drink from a

Bottle with a teat ☐☐ Feeding cup with lid ☐☐ Cup without a lid ☐☐

11. To help us decide on the correct amount of fluids for drinks, please fill your baby's **cup** with water to the usual level, then empty the water into a measuring jug and record here.

Fluid Ounces ☐☐ (fl. oz.) or Millilitres ☐☐☐ (ml) Doesn't use a cup ☐

12. Baby's first solid foods are normally smooth purees.
At what age did you start giving foods with a lumpier texture e.g. noodles? ☐☐ weeks or ☐ ☐ months

☐ haven't introduced yet

13. At what age did your baby start eating finger foods e.g. toast? ☐☐ weeks or ☐☐ months

☐ haven't introduced yet

14. What age was your baby when you stopped breast feeding? ☐☐ weeks or ☐ ☐months

☐ still breast feeding

15. Does your baby have definite likes and dislikes as far as food is concerned?

☐ no, will eat almost anything ☐ yes, quite choosy ☐ yes, very choosy

16. Do you feel that you have had difficulty feeding your baby since he/she was eight months old

☐ Yes, great difficulty ☐ Yes, some difficulty ☐ Yes, occasional difficulty

☐ No, no difficulty

17. Has your baby at any time in the past 2 months:

	Yes, worried me greatly	Yes, worried me a bit	Yes, but did not worry me	No, did not happen
a) Not eaten sufficient amount of food	☐	☐	☐	☐
b) Refused to eat the right food	☐	☐	☐	☐
c) Been choosy with food	☐	☐	☐	☐
d) Over-eaten	☐	☐	☐	☐
e) Been difficult to get into an eating routine	☐	☐	☐	☐
f) Any other problems with feeding (please describe)	☐	☐	☐	☐

____

____

18. In your opinion, does your child eat differently than other children of the same age? Yes ☐ No ☐

If yes, please describe____

The Brighton's Breast-fed Babies Study

Food Diary

Date

____

Day of the week

____

Study number

How to fill in the diary

Now that your baby is eighteen months old we would like to look at his or her diet again. Thank you very much for helping us by filling in these food diaries.

Please could you record everything that your baby has by mouth for 3 days this week. There are 3 diary booklets, one for each day, with different pages for you to record what your baby eats and drinks. Start each diary booklet when you get up in the morning and fill in everything your baby eats and drinks for a 24-hour period until the same time the next day. Please choose one weekend day and two weekdays. They need not be one after the other. If any day is likely to be very difficult or unusual choose another day. It is very important that you do not change what your baby normally eats and drinks just because you are keeping this record.

Try to fill in the pages as you go through the day as this is much easier and more accurate than trying to remember at the end of the day. If someone else looks after your baby for some of the time it would be most helpful if they could fill in the food given in the parts of the day when your baby is with them.

When recording the food given please include the brand name (if known), portion size (using feeding jar size, cup or spoon size, weights from labels), any additions to the food (fats, oils, sugar/sweeteners, sauces, salt, pepper etc.) and cooking methods (fried, grilled, micro-waved, roasted). We have given you a booklet "How to describe your baby's food and drink" which shows you the sort of detail we need, and there are also some examples given in the diary.

Recipes/homemade dishes

If your baby has eaten any **homemade dishes** e.g. chicken casserole, please record the name of the recipe, ingredients with amounts (including water or other fluids) for the whole recipe, the number of people the recipe is for, and the cooking method. Record how much of the whole recipe your toddler ate.

Take-aways and eating out

If your baby has eaten **take-aways** or **made up dishes not prepared at home** such as at a restaurant or a friend's house, please record as much detail about the ingredients as you can e.g. spaghetti with mince, onion and tomato sauce.

Labels/wrappers

These are an important source of information for us. It helps us a great deal if you enclose, in the plastic bag provided, labels from all ready meals, labels from foods of lesser known brands and also from any supplements your baby takes.

Our researcher will go through the food diaries with you at her next visit. If you can't manage all 3 days then one or two complete diaries are still very useful to us.

Please complete the inside pages as you go through the day and this page at the end of the day.

Please put a ☑ in the box beside your answer

1. Did anyone else look after your baby during any mealtimes today?

No ☐ Yes ☐ If yes please indicate which meals.

Breakfast ☐ Midday meal ☐ Evening meal ☐

Who looked after your baby at these mealtimes? Partner ☐ Relation ☐

Friend ☐ Nanny or minder ☐ Nursery ☐ Playgroup ☐ Other ☐ ____________________

2. As far as you know was all the food and drink taken by your baby during this 24 hours recorded?

Yes ☐ Not sure ☐ No ☐

3. Was the food and drink for this 24 hours fairly typical of your baby?

Yes ☐ No ☐

If no, please describe how it differed from normal and if your baby was unwell during this 24 hours.

____

Has anyone else looked after your baby today? Yes ☐ No ☐

Please record any parts of this 24-hour period when someone else looked after your baby.		
Start Time (e.g. 9.30am)	Return Time (e.g. 12.00 noon)	Time elapsed

Record here any FOODS your baby EATS in this 24 hours.

See following sections to record milk-feeds and other drinks.
Please start each food on a new line

Each day is divided into time intervals, from before breakfast to the evening meal and through the night. Please find the appropriate time interval and write in the time when your baby eats something. Don’t forget to include snacks like sweets, chocolate, biscuits, crisps, ice cream or fruit and spreads e.g. margarine .Also include any sugar added to cereals or drinks.

YOUR BABY’S FOOD

Time interval	**Time**	**Description of food and amount eaten** **Please give as much detail as possible including the raw ingredients and cooking method of all homemade foods. Also please give us a full description, including the brand and flavour, of other meals given with any liquid, sauces or foods you added.**
Before Breakfast		
Breakfast		
Mid-morning		

Please tell us if you have used a different type of bread or margarine/butter from that recorded on the GENERAL FOOD AND DRINK QUESTIONS *questionnaire.*

Foods continued

Record here all the FOODS your baby EATS, from lunchtime and through the rest of the day and evening, along with how much he or she ate.

See following sections to record milk-feeds and other drinks.

Time interval	**Time**	**Description of food and amount eaten** **Please give as much detail as possible including the raw ingredients and cooking method of all homemade foods. Also please give us a full description, including the brand and flavour, of other meals given with any liquid, sauces or foods you added.**
Lunch		
Tea Between lunch and evening meal		
Evening meal		
Later evening and through the night		

Please tell us if you have used a different type of bread or margarine/butter from that recorded on the GENERAL FOOD AND DRINK QUESTIONS *questionnaire.*

If your baby has a MILK-FEED at any time during this 24 hours, please fill in this section.

Time	**Bottle/cup feed of milk** How much did your baby drink? Fluid ounces (fl. Oz.) or Millilitres (ml)	**Breast feed** Number of minutes the feed lasted

Whatever other DRINKS your baby has, including water, in this 24 hours can be recorded here.
Please find the appropriate time slot and then record the time your baby has a drink.
Note the type of fruit juice, squash or fizzy drink etc. Please also include and vitamins or medicines on this sheet.

Time interval	**Time**	**Full description and/or brand of drink. Please remember any vitamins or medicines.**	**Did you dilute with water? yes/no**	**How much concentrate did you use?**	**Did you add sugar? Number of teaspoons?**	**How much did your baby drink?**
Before breakfast						
Breakfast						
Mid-morning						
Lunch						
Tea – between lunch and evening meal						
Evening meal						
Later evening – and through the night						

Please write any notes, comments or questions here.

Appendix 2

Supporting Documents for The GO-CHILD Study

My baby's nine-month feeding questionnaire

Now that your baby is nine months old, we would like to ask you some questions about their feeding.

All babies are different and have different likes and dislikes. There are no right or wrong answers, please just give the answer that is most accurate in your opinion.

If you made notes about what your baby has been eating and drinking in your baby's 'Red Book'/health book you might find it helpful to look at them when answering this questionnaire.

Thank you for taking the time to answer our questions. We hope that your answers will help us to advise parents in the future about the best way to feed their babies.

Name of Child: .... **Bar Code Sticker**

CHI / NHS Number: ....

What date did you fill in this questionnaire: ______________ MONTH ________ DAY

Questionnaire filled in by Mother ☐ Father ☐

Other (please describe relationship to baby) ☐

____

Barcode sticker

Milk feeds

1 Has your baby ever had any of the following milk types since birth? Please include milk used for mixing with solids or used in cooking

	No	**Yes**	Age started (months)
a) breast milk	☐	☐	…………………..
a) ordinary formula	☐	☐	…………………..
b) follow-on formula	☐	☐	…………………..
c) ordinary cow's milk	☐	☐	…………………..
d) goat's/sheep's milk	☐	☐	…………………..
e) hypo-allergenic formula	☐	☐	…………………..
f) soya milk (please specify)	☐	☐	…………………..
g) other milk (please specify)	☐	☐	…………………..

____

2. Did you ever put your baby to the breast, even for a single feed?

Yes ☐ No ☐ (If no then please go to question 4)

3. Are you still breastfeeding?

Yes ☐ If yes, roughly how many times in a typical day ………………..

No ☐ If no, how old was your baby when they had their last breastfeed

…….. months or………weeks

4. During the past week how many bottle or cup feeds of infant formula or other milks (not breast milk) has your baby had on a typical day? Please answer for each different milk, excluding breastmilk. Please give names of milks, e.g. SMA 1, Aptamil Hungry Milk 2, Heinz Nurture Follow On, Hipp Organic Good Night Milk etc.

No bottle or cup feeds ☐ (please skip to question 6)

Type of milk (e.g. cow's milk, name of formula)	How many bottles/cups per day	Amount drunk from each bottle/cup
		………..ml or ……….oz
		………..ml or ……….oz
		………..ml or ……….oz

5. Has your baby regularly drunk any other brands or types of formula milk since they were born? Please give names and brands and age used if possible.

Name of formula milk	Age started	Age stopped
	………...wks or ………mo	………...wks or ………mo
	………...wks or ………mo	………...wks or ………mo
	………...wks or ………mo	………...wks or ………mo

6. Do you ever add cereal to your baby's bottle?

Yes always ☐ Yes often ☐ Yes sometimes ☐ No never ☐

7. Do you add sugar to your baby's food or bottle?

Yes always ☐ Yes often ☐ Yes sometimes ☐ No never ☐

Solid foods and other drinks

8. If you buy ready-prepared baby foods how often do you choose organic baby foods?

Never ☐ Sometimes ☐ Most of the Time ☐ All of the time☐

Don't use ready made ☐

9. How many meals of solid or semi-solid foods does your baby eat each day now?

____

10. When did your child first start having meals with lumps in or finger foods (rather than purees)?

---------- months or ---------------weeks

Hasn't started meals with lumps or finger foods yet ☐

11. Has your baby ever had any of the following foods and drinks?
Please tick no or yes as appropriate. If yes, please tell us how old your baby was when they started having the food and how often they are having it nowadays (if they have the food once a week or more, please fill in the column for times a week, if it is less than once a week, please fill in the column for times a month).

	No	**Yes**	**Age Started (months)**	**Times a week**	**Times a month**
Dry baby foods					
Plain baby rice					
Flavoured baby rice					
Other cereal e.g. porridge					
Gluten free rusks					
Normal baby rusks					
Dried packet meal containing meat					
Dried packet meal containing fish					
Dried veg/pasta/rice based meals					
Dried desserts					
Jars, tins and pots of baby foods					
Breakfast meals e.g. porridge					
Meals containing meat					
Meals containing fish					
Vegetable, pasta or rice based meals					
Milk or cereal based dessert e.g. rice pudding, yoghurt, chocolate pudding					
Pure fruit puree					
Other fruit desserts					
Family foods					
Weetabix-type breakfast cereal					
Other breakfast cereals					
Potatoes, boiled/mashed					
Chips or roast potatoes, potato waffles etc.					
Carrrots					
Other root veg (e.g. sweet potato, swede, parsnip)					
Rice					
Pasta					
Chicken or turkey					
Sausages or burgers					
Red meat, beef, lamb, pork					

	No	Yes	Age Started (months)	Times a week	Times a month
Liver, kidney, heart etc.					
White fish e.g. cod, haddock, fish fingers etc.					
Tuna					
Other oily fish eg. salmon, trout, sardines, mackerel etc					
Eggs or quiche					
Cheese					
Baked beans					
Other beans and pulses e.g. lentils, chickpeas, butter beans, humus etc.					
Peas and green beans					
Green leafy veg e.g. broccoli, cabbage, sprouts					
Tinned tomatoes					
Other cooked veg					
Raw veg and salad					
Yoghurt/fromage frais					
Milk puddings e.g. custard, rice pudding, mousse					
Cooked fruit					
Banana					
Oranges, satsumas, kiwis, strawberries					
Other fresh fruit					
Soya/vege burgers and sausages etc.					
Quorn					
Peanuts or peanut butter					
Other nuts					
White bread or toast					
Brown/wholemeal bread or toast					
Crackers/breadsticks					
Biscuits					
Cakes, buns, pastries					
Crisps or similar snacks					
Chocolate					
Sweets					
Ice cream/ice lolly					
Drinks					
Baby fruit juice					
Other fruit juice					
Ribena/hi-juice blackcurrant squash (not low sugar)					
Other squash (not low sugar)					

	No	**Yes**	**Age Started (months)**	**Times a week**	**Times a month**
Ribena lite/low-sugar blackcurrant squash					
Other low-sugar squash					
Fizzy drinks					
Tea					
Water on its own					

12. Has your baby had any other foods or drinks that we have not included? Please list any other foods and drinks below.

Name of food or drink	Age Started (months)	Times a week	Times a month

13. How much food does your baby normally eat at each meal? There are photographs of different jars and portion sizes at the end of the question to help you answer this.

a) Savoury foods e.g. meat, vegetables, potatoes, pasta etc.

What size is the jar? Small (stage 1) ☐ OR Large (Stage 2) ☐

Proportion of a jar ¼ or less ☐ $^{1}/_{3}$ ☐ ½ ☐ $^{2}/_{3}$ ☐ ¾ ☐ whole jar ☐

OR number of teaspoons of food _______

OR number of "ice cubes" _______

OR
Proportion of an "adult serving" $^{1}/_{8}$ or less ☐ $^{1}/_{6}$ ☐ ¼ ☐ $^{1}/_{3}$ ☐ ½ or more ☐

b) Pudding eg. rice pudding, yoghurts or fruit purees

What size is the jar? Small (stage 1) ☐ OR Large (Stage 2) ☐
Proportion of a jar ¼ or less ☐ $^{1}/_{3}$ ☐ ½ ☐ $^{2}/_{3}$ ☐ ¾ ☐ whole jar ☐

OR number of teaspoons of food _______

OR number of "ice cubes" _______

OR
Proportion of an "adult serving" $^{1}/_{8}$ or less ☐ $^{1}/_{6}$ ☐ ¼ ☐ $^{1}/_{3}$ ☐ ½ or more ☐


1 teaspoon of food


2 teaspoons of food


4 teaspoons of food

Shown in 16.5cm/6.5 inch diameter bowl

Stage 1 jar Stage 2 jar

14. How old was your baby when solid foods were first introduced?

………months or ………weeks

15. What was the first solid food he/she regularly ate?

……………………………………………………………………………….

16. Does your baby drink out of a cup?

Yes, usually ☐ Yes, sometimes ☐ No, not at all ☐

17. Since your baby was born, have you given him/her any vitamins or mineral drops, including iron and fluoride drops

Yes ☐ No ☐

If yes, please state which

	Yes	No	Age started Weeks	or Months
Healthy Start Vitamin A,C,D drops	☐	☐	………..	…………
Iron drops	☐	☐	………..	…………
Fluoride drops	☐	☐	………..	…………
Other, please describe	☐	☐	………..	…………

……………………………………………………..

18. Has your baby ever been given supplements of fish oil, hemp oil, evening primrose oil or other oils?

No ☐ Yes ☐

If yes	Age started Weeks	or Months
Name of oil………………………………………………………	…………….	………………..

19. Has your baby ever regularly consumed probiotics, either in yoghurt, or in another food or drink, or as a supplement?

Yes	No	Age started Weeks	or Months
☐	☐	……………	………………

20. Is your baby being brought up as a vegetarian or vegan?

Yes, vegetarian ☐ Yes, vegan ☐ No ☐

21. Does your baby have definite likes and dislikes as far as food is concerned?

☐ no, will eat almost anything ☐ yes, quite choosy ☐ yes, very choosy

22. How much does your baby like the following foods?

	Likes this very much	Quite likes this	Does not really like this	Will not eat this	Has not tried this
a) Meat	☐	☐	☐	☐	☐
b) Fruit	☐	☐	☐	☐	☐
c) Vegetables	☐	☐	☐	☐	☐

23. Do you feel that you have had difficulty feeding your baby since he/she was eight months old

☐ Yes, great difficulty ☐ Yes, some difficulty ☐ Yes, occasional difficulty ☐ No, no difficulty

24. Since he/she was six months old has your baby at any time

	Yes, worried me greatly	Yes, worried me a bit	Yes, but did not worry me	No, did not happen
a) Not eaten sufficient amount of food	☐	☐	☐	☐
b) Refused to eat the right food	☐	☐	☐	☐
c) Been choosy with food	☐	☐	☐	☐
d) Over-eaten	☐	☐	☐	☐
e) Been difficult to get into an eating routine	☐	☐	☐	☐
f) Any other problems with feeding (please describe)	☐	☐	☐	☐

____

____

THANK YOU AGAIN FOR YOUR HELP IN FILLING IN THIS QUESTIONNAIRE.
If you would like more information about the study, please contact Dr Konnie Basu at k.basu@bsms.ac.uk