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University College Cork, Ireland

Early Milk Diet Of Infants & The Effect On Their Body Composition And Growth And Development In The First Two Years Of Life

A thesis submitted to the National University of Ireland, Cork for the degree of Doctor of Philosophy in the Paediatrics and Child Health, School of Medicine, College of Medicine and Health

November 2018

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Statement of contribution

This thesis is the result of research performed on data collected as part of the Cork BASELINE Birth Cohort Study, in addition to data from the SCOPE Ireland Study.

From 2009 to 2012 (1st December 2009 to 30th March 2012) I worked as a research midwife as part of the Cork BASELINE Birth Cohort research team. In October 2012 I began a period of research, allowing me to analyse the growth and feeding data collected as part of the study protocol. This analysis has formed the foundation for this thesis.

My role within the Cork BASELINE Birth Cohort Study research team included recruiting, and consenting, participants to the study, establishing them on the study's database and undertaking follow-up appointments. I also undertook administrative duties in arranging follow-up assessments, which took place at two, six, 12 and 24 months. During follow-up assessments I outlined the purpose and activity of each appointment and confirmed parental consent to continue with each assessment, administered parental questionnaires, performed body composition and anthropometric assessments and where needed provided eczema care and allergy testing. I undertook the majority of follow-up appointments at two months and was one of two lead research nurse/midwives to undertake the remaining assessments at six, 12 and 24 months. I also assisted the research midwife with the SCOPE Ireland Study in obtaining newborn body composition and anthropometric assessments. I had co-responsibility for the maintenance and calibration of the PEA POD® Body Composition Tracking System. For the 24-month neurodevelopment assessment I assisted the research psychologist with ensuring parents completed the age appropriate questionnaires. I also scored questionnaires and neurodevelopment assessments undertaken by the research psychologist. I developed and reviewed Standard Operating Procedures for the Cork BASELINE Birth Cohort Study.

Prior to any data being exported for analysis, I developed and performed a data validation assessment, using Microsoft Excel 2007, to ensure the completeness and accuracy of all data points for the full Cork BASELINE Birth Cohort Study database. I then generated, tracked, answered and closed data queries on the database. I also extracted, labelled and coded all data. I undertook all data analysis, with support and guidance from Dr. Darren L Dahly (principal statistician with UCC-Clinical Research Facility and Senior Lecturer with the School of Public Health, UCC).

The work I am submitting is my own and has not been submitted for another degree, either at University College Cork or elsewhere. All external references and sources are clearly acknowledged and identified within the contents. I have read and understood the regulations of University College Cork concerning plagiarism.

Hazel A Smith

Hazel A Smith

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I am indebted to my friends and family who have helped me in completing my thesis and for their mental support. During this PhD I lost some very important people in my life but I would like to dedicate my thesis to my uncle Frank. We were all so incredibly lucky to have such an amazing uncle. Frank had so much strength, love and a brilliant memory for sharing stories. Frank was taken from us way too soon but his memory never fades.

Abstract

Background: Nutrition in the first few months of life has important effects on long-term growth. The aim of this PhD was to investigate the effect of an infant's milk diet (both formula and breastmilk intake) in the first two months of life on body composition at two months of age, growth in the first two years of life and neurodevelopment at two years; and to examine whether breast- and formula-fed infants differ at birth, confounding the true effect of breastfeeding.

Methods: Secondary data analysis of the feeding patterns, growth and development of children in the Cork BASELINE Birth Cohort Study. Descriptive and multivariate (multi-linear and logistic regression) analysis was employed.

Results: Admission to the neonatal intensive care unit had the greatest negative impact on exclusively breastfeeding at two months (adjusted odds ratio = 0.20 (95% CI 0.05, 0.83)).

Nearly twice as many exclusively formula-fed infants experienced early rapid growth (ERG) at two months compared to exclusively breastfed infants, n=87 (30%) vs n=56 (16.9%), respectively. Infants that experienced ERG saw an increase in their weight-for-height (wfh) z-score at 24 months compared to infants that did not experience ERG, β=0.39 (95% CI 0.19, 0.54).

Breastfed infants had a higher mean(SD) birthweight to formula-fed infants, 3.56(0.42)kg versus 3.46(0.44)kg, respectively. However, breastfed infants had a lower mean(SD) percentage fat mass at birth compared to formula-fed infants, 10.01(3.71)% versus 12.05(4.06)%.

Conclusion statement: By two months of age few Irish infants are exclusively breastfed. Formula supplementation and admission to the neonatal intensive care unit in the maternity hospital shortened breastfeeding duration. Formula feeding increased the odds of ERG and experiencing ERG at two months increased a child's wfh z-score at 24 months. Breastfed infants were different in growth and body composition at birth in our cohort.

Research Outputs From Thesis

Conference Presentations

Dahly, D.L., Li, X., **Smith, H.A.**, Khashan, A.S., Murray, D.M., Kiely, M., O'B Hourihane, J., McCarthy, F.P, Kenny, L.C., Kearney, P (July 2018) *Associations between maternal lifestyle factors and neonatal body composition in the Screening for Pregnancy Endpoints (Cork) cohort study* Oral presentation at European Congress of Epidemiology in Lyon, France

Hazel A Smith, Jonathan O'B Hourihane, Mairead Kiely, Louise C Kenny, Patricia Leahy-Warren; Deirdre M Murray (May 2017) *Exclusive Formula Feeding Increased the Risk of Early Rapid Growth in Infancy* Oral presentation at Our Lady's Children's Children, Crumlin Research and Audit Day in Dublin, Ireland

Smith, H. A., O'B Hourihane, J., Kenny, L. C., Kiely, M. & Murray, D. M (November 2015) *Are Formula-Fed Infants Following European Recommendations?* Poster presentation at the 8th Irish Research Nurses Network Annual Conference in Dublin, Ireland

Smith, H. A., Leahy-Warren, P.& Murray, D. M. (December 2014) *Feeding patterns in infants and the effects on early growth/development: Data from the Cork BASELINE Birth Cohort Study* Oral presentation at the National Children's Research Centre Research Symposium in Dublin, Ireland

Smith, H. A., Leahy-Warren, P. & Murray, D. M. (June 2014) *Systematic review of infant formulae feeding practices* Poster presentation at the 30th ICM (International Confederation of Midwives) Triennial Congress 2014 in Prague, Czech Republic

Smith, H. A., Leahy-Warren, P.& Murray, D. M. (May 2014) *Formula protein content and neurodevelopmental outcomes* Oral presentation at the National Children's Research Centre Research Symposium in Dublin, Ireland

Smith, H. A., O'B Hourihane, J., Kenny, L. C., Kiely, M. & Murray, D. M (February 2014) *Formula Protein Content And Body Composition From Birth To 2months Of Age: Data From The Cork Baseline Birth Cohort Study* Oral presentation at the 2nd International Conference on Nutrition and Growth 2014 in Barcelona, Spain

Smith, H. A., O'B Hourihane, J., Kenny, L. C., Kiely, M. & Murray, D. M (February 2014) *Are Formula-Fed Infants Following European Recommendations?* Poster presentation at the 2nd International Conference on Nutrition and Growth 2014 in Barcelona, Spain

Smith, H. A., Leahy-Warren, P.&Murray, D. M. (December 2013) *Formula protein content and early growth* Oral presentation at the National Children's Research Centre Research Symposium in Dublin, Ireland

Smith, H. A., O'B Hourihane, J., Leahy-Warren, P.&Murray, D. M. (May 2013) *Milk feeding patterns of infants and the impact on growth, neurodevelopmental outcomes and atopic disease* Oral presentation at the National Children's Research Centre Research Symposium in Dublin, Ireland

Publications

Smith, H. A., O'B Hourihane, J., Kenny, L. C., Kiely, M., Leahy-Warren, P., Dahly, D.L. & Murray, D. M (accepted for publication in March 2019) Set up to fail: the body composition of formula fed infants differs from breastfed infants at birth prior to milk feeding exposure J*ournal of Developmental Origins of Health and Disease*

Smith, H. A., O'B Hourihane, J., Kenny, L. C., Kiely, M., Leahy-Warren, P.& Murray, D. M (submitted – under reveiw) Effect of exclusive formula feeding, and formula content, on early rapid growth *Maternal and Child Health*

Dahly, D.L., Li, X., **Smith, H.A**., Khashan, A.S., Murray, D.M., Kiely, M., O'B Hourihane, J., McCarthy, F.P, Kenny, L.C., Kearney, P (2018) Associations between maternal lifestyle factors and neonatal body composition in the Screening for Pregnancy Endpoints (Cork) cohort study *International Journal of Epidemiology* 47(1): 131–145

Smith, H. A., O'B Hourihane, J., Kenny, L. C., Kiely, M., Leahy-Warren, P.& Murray, D. M. (2016) Infant formula feeding practices in a prospective population based study *BMC Paediatrics* 16:205

Smith, H. A., O'B Hourihane, J., Kenny, L. C., Kiely, M., Murray, D. M & Leahy-Warren, P. (2015) Early life factors associated with the exclusivity and duration of breast feeding in an Irish birth cohort study *Midwifery* 31(9): 904-911

Chapter 1: Introduction

1.1 Brief Introduction with Aims of Thesis

International guidelines promote exclusive breastfeeding for the first six months of life[1, 2] but the majority of infants receive infant formula (either with breastmilk or are exclusively formula-fed)[3]. For infants that are given infant formula it is recommended that they receive a whey-based infant formula (unless medical advised otherwise)[4-7]. However, there is range of infant formula with different protein composition and it is unknown if infants are being whey-based infant formula or non whey-based infant formula fed.

The promotion of growth in the early postnatal is primarily driven by nutrition and in the first few months of life an infant's diet is milk based[8]. Infant growth, especially during the first two months of life, can have a life-long effect on health. One of the main potential risks of rapid growth, driven by infant diet, is later onset overweight and obesity[9]. Growth is not the only part of infant development to go through rapid changes. Rapid changes in neurodevelopment also occur during this time and there is research to show that both diet and growth can influence neurodevelopment progress in infancy.

Previous research examining the effect of breastfeeding, versus infant formula, on size, growth and neurodevelopment have often being confounded by including infants that receive both breastmilk and infant formula[10]. Another limitation in exploring diet and growth is that nearly all studies include birth weight but not birth composition. Weight is the total mass measurement of a person's fat and fat free masses. Weight, as a measurement, does not inform a healthcare professional if an infant has an excessive fat accumulation. Therefore, it remains unknown if infants who subsequently go on to be 'breastfed' or 'formula-fed' differ at birth.

Given this background the aim of my thesis was to use a large well characterised birth cohort study to:

1) Determine the rates of exclusive breastfeeding in the first year of life in an Irish population, and the maternal, paternal and infant factors that influence breastfeeding exposure.
2) Determine the use of whey-based and non-whey based infant formula in the first year of life and the maternal, paternal and infant factors that influence parental choice of formula.
3) Compare the growth of exclusively breastfed to exclusive formula fed infants in the first two years of life
4) Determine the effect of early milk diet of infants (comparing exclusively formula fed to breastfed) infants on their neurodevelopment at two years of life
5) Determine if body composition differences exist at birth in infants who proceed to be exclusively breastfed compared to those exclusively formula fed.

1.2 Full Introduction

In July 2014, the World Health Organization (WHO) hosted its first meeting of the Commission on Ending Childhood Obesity (ECHO). In their 2016 publication the WHO estimated that about 41 million children under the age of 5 years were either overweight or obese[11]. Previous estimates, in 1990, had reported that 32 million children, aged five years or less, were likely to be overweight/obese. Future projections of this trend, if unchanged allowed the authors to predict that by 2025 70 million children under five years of age would be overweight or obese[12].

Ireland has one of the highest international rates of obesity[13]. Findings from the Growing Up in Ireland (GUI) project showed that one in four Irish children (aged three years) were either overweight or obese[14]. The rate of childhood obesity, for this cohort of children, remained the same when followed up at five years of age[15]. One of the most recent papers examining the longitudinal effect of childhood obesity was a meta-analysis published in 2017[16]. The authors reported that childhood obesity significantly increased the odds of obesity in adulthood (OR 1.45, 95% CI: 1.20, 1.76), compared to children who were not obese.

The consequences of childhood obesity include increased risks of developing metabolic syndrome, musculoskeletal disorders and certain types of cancer. These increased risks and disorders also lead to increased costs to the health sector and negatively impact on the quality and quantity of life for the overweight or obese person[17]. In 2013 the Irish government launched the Healthy Ireland framework (http://health.gov.ie/healthy-ireland/)[18],[19] . The aim of the framework is to improve the health of people in Ireland and one of the commitments is to tackle Ireland’s current rate of childhood obesity. The Healthy Ireland framework plans to address health inequalities from the antenatal period through to old age[20].

Current research has shown that events and exposures, which influence growth, from conception up to two years of age can effect life-long health[21].. A number of systematic reviews and meta-analyses have shown that rapid growth in infancy is associated with an increased risk of later obesity[9, 22-26]. Current evidence has suggested that the first two months is a particularly important period in the modifying the risk of obesity[21, 27-31];[32]. Although different definitions of rapid growth have been used, the most commonly reported definition of rapid growth, in research studies, is an upwards change of 0.67 standard deviation (SD) or greater in the weight-for-age z-score during infancy, as this equates to one major centile line.

As a rule of thumb it is said infants double their birthweight by six months of age and triple their birthweight by their first birthday[33]. Most of the weight gain between birth and six months is driven by the weight gain achieved in the first two months of life. In the first two months of life, weight gain is about 25g to 35g per day and length and head circumference both generally increase by two centimetres per month[33, 34]. After the first two months of life an infant gains about 12g to 21g of weight per day and their head circumference increases by one centimetre per month[34, 35]. Positive increases in z-scores indicate growth which has surpassed the expected gains in growth. The distance between two major centile lines is 0.67SD, so for infants whose growth rate is faster than what would be expected and experience upward crossing of centile lines they will show a positive change of 0.67SD or greater in the relevant z-score. The hypothesis built around the finding that early rapid growth is a risk factor for the later metabolic syndrome, including obesity, was coined the Growth Acceleration Hypothesis[36] by Professors Singhal and Lucas, from the Institute of Child Health in London.

In order to determine if a child is growing at a healthy and desired rate reference data are needed to make growth charts to allow accurate comparisons and definitions. Reference data provide expected normative measures of size and growth which can be stratified by single or multiple characteristics – gestational age at delivery, maternal parity, infant sex, singleton/ multiple pregnancy, infant ethnicity or maternal country of birth etc. Reference data can also be made available for the general public[37, 38] or specific sub-groups[39-41]. From the 1st of January 2013 the Irish Department of Health recommended that all newborn infants, born ≥ 32 gestational weeks', have their growth monitored using the United Kingdom-World Health Organization (UK-WHO) Growth Standards charts[42]. The WHO Growth Standards originate from their Multicentre Growth Reference Study (MGRS), which collected longitudinal anthropometric data on healthy term, exclusively breastfed, infants. To-date over 125 countries have adopted the WHO Growth Standards to monitor child growth and health[43].

For today's researchers investigating the effect of environmental, dietary and hereditary exposures on infant and childhood growth, interest has grown in estimating the body composition of participants. Two compartment body composition assessments, in comparison to weight, provide information on the variation of fat mass (FM) and fat free mass (FFM)[44, 45]. New-born infants' body composition is an ideal baseline measurement, as it details the FM and FFM prior to any postnatal exposures and reflects the intra-uterine environment. Additional body composition measurements after birth enable researchers and clinicians to track the infant body composition changes against environmental, dietary and hereditary exposures. One of the benefits of these repeated assessments is that researchers have more opportunities to evaluate the effect of different nutritional exposures on infant body composition and how this impacts on their risk of later onset obesity[46].

There are various body composition methods available to measure both neonatal and infant body composition. It should be noted that the amount of detail obtained from the body composition measurement will depend on the method used, i.e. 2-compartment model divides the measured subject into FM and FFM only. Three or greater compartment models measure FM and divide FFM into its various components (which is the body's total mass minus the fat mass and includes bone, water and muscle). Nutritional, metabolic and anatomical models exist which examine body composition and fat distribution in a variety of ways[47].

In studying an infant and child's growth and/or body composition it is important to understand what determinants influence this growth in early life. The determinants of growth include genetic, hormonal, environmental and dietary factors. One of the primary environmental factors that influence weight gain during the first two years of life, and especially in the first two months of life is the infant's nutritional intake[48]. Human breastmilk is viewed as the biologic nutritional norm for infants and is seen as the gold standard for infant nutrition. This is reflected in the opening line of The Lancet's 2016 Breastfeeding series 'Breastmilk makes the world healthier, smarter, and more equal'[49]. Findings from this series reported that breastfeeding had both short-and long-term positive effects on child's health and development[50]. Human breastmilk is species-specific nutrition which supports the growth, development and health of infants. Infant formula is a breast-milk substitute[51]. Infant formulae, in comparison to breastmilk, have higher levels of energy and protein[52].The potential effect of high protein intake (compared to breastfeeding) on growth was first examined in 1989 by Dr Irene E Axelsson and colleagues from the Department of Paediatrics, Malmö General Hospital, Sweden[53]. Results showed that infants assigned to an infant formula with 1.8g of protein per 100ml had higher weight and length gain compared to both breastfed and infants assigned to the lower protein content (1.3g/100ml) infant formula. In 1995 Marie Françoise Rolland-Cachera and her colleagues in France first suggested the protein-adiposity hypothesis, i.e. that a high protein intake predisposed infants to later onset obesity[54]. In 2009 the European Union funded Childhood Obesity Project[52] put forward the Early Protein Hypothesis. The Early Protein Hypothesis speculates that infants who are fed an infant formula with protein levels which exceed their metabolic requirements are at an increased risk of later-onset overweight and obesity due to the rapid growth that they experience in infancy[52]. This hypothesis is, in many ways, an extension of the Growth Acceleration Hypothesis. Both state that rapid growth in infancy is a risk factor for the components of the metabolic syndrome. The Early Protein Hypothesis speculates that it is the protein content of the infant formula which drives this rapid growth. The four RCTs[54-57] that have investigated the effect of the protein content in infant formula found a direct relationship with protein intake and later onset overweight and obesity.

Out of the studies that examined Early Protein Hypothesis none of the observational studies exclusively examined the effect of the protein content in infant formula as they included protein intake from all dietary sources. However, all RCTs did examine the effect of early protein intake from infant formula (but not from commercially available infant formula) and not other dietary sources on later onset overweight and obesity. The studies each had infants exposed to infant formulae with 'high' and 'low' levels of protein. The cut off level for high and low protein content was determined by the RCTs themselves and all studies had breastfeeding infants as the control group. The age of infants when the protein exposure was measured ranged from two months[55, 56] to two years of age[54] and the endpoint of the studies varied from six months[53] to 10 years of age[58]. Another limitation is that the studies did not compare other differences in formula composition and protein is only one of the three major macronutrients. The different fat and carbohydrate content in infant formulae could also influence infant growth[54, 56, 59-62].

The RCT from the European Union Childhood Obesity Project[56] and RCT undertaken in Chile[62] were the only two studies that determined their sample size from power calculations. Only the Chilean RCT was powered to detect weight difference but this was just for the primary endpoint measured at three-six months postnatal. Sample sizes varied across all the studies, ranging from 30[53] to 934[56] participants. One of the main limitations of studies with small sample sizes is that you are unable to make firm conclusions about the results. Therefore it is difficult to determine if the effect seen is by the intervention or by chance alone[63].

Protein levels and protein type differ across infant formulae. There are several types of milk proteins but whey and casein are the two major milk proteins. Breastmilk is predominantly whey-based and is easier for infants to digest compared to casein proteins[64]. It is for these reasons that, internationally[5-7, 65-67], whey-based infant formula is the recommended type of infant formula for non-breastfed infants. Whey-based infant formula also contain leucines levels that are similar to those found in breastmilk[68]. In comparison, non whey-based infant formula can have levels one and a half times to the levels found in breastmilk. Leucines are branched chain amino acids (BCAA) and research has suggested that BCAAs are important mediators for metabolic signalling[69, 70] via the mammalian target of rapamycin complex 1 (mTORC1) pathway[68, 71-79]. The mTORC1 is a nutrient-sensitive kinase and is a key regulator of cell co-ordination, growth and proliferation for muscle, adipose tissue (white and brown fat), pancreas (β-cells and insulin secretion) and liver (IGF-1 production and also contributes to liver regeneration). The mTORC1 signalling system is primarily activated through protein composition provided by milk and branched-chain amino acids (principally leucine). Digesting more leucines than what is needed can over stimulate the mTORC1 pathway and thereby promote cell growth and adipogenesis (the process where the cells differentiate into adipose tissue)[80]. Infants who are formula-fed have a greater intake of leucines, which may promote faster growth[81].

Growth is not the only aspect of child development to go through rapid changes during the early postnatal period and therefore it should not be examined in isolation. Although the causes of neurodevelopmental delay are mutli-complex and nutrition or growth alone do not stimulate brain development they are key factors. Head circumference and brain volume are correlated to each other and during the early postnatal period brain growth is rapid[82, 83]. The postnatal period is a crucial period for white matter development and myelination. Myelination is complete by approximately 2 years of age. Rapid changes in brain complexity and connectivity occur during this time, with life-long consequences[82, 83]. Evidence, primarily from studies examining infants born with intrauterine growth restriction (IUGR), pre-term small-for-gestational age (SGA) and pre-term, have reported that limited physical growth is associated with poorer neurodevelopment outcomes compared to children with average physical growth [84-89].

Results from a Cochrane review examining the topic found that increased protein intake (≥ 3.0 g/kg/d but < 4.0 g/kg/d) compared to < 3.0 g/kg/d of protein promoted accelerated weight gain in low-birth weight infants (<2.5kg) but the evidence was inconclusive on whether this also resulted in improved neurodevelopment outcomes[84]. Other studies have shown that insufficient protein intake can have a negative effect on head circumference size and growth and therefore also on neurodevelopment[90].

The promotion of growth in the early postnatal period is primarily driven by nutrition and nutrition during this period is either controlled by healthcare professionals or parents. Both growth and nutrition are influencing factors for an infant's neurodevelopmental outcomes. This literature review so far has shown that there is evidence to support that an infant's body size and growth are primarily driven by dietary exposures during infancy[48] and that altered growth patterns are associated with impaired neurodevelopment outcomes[90]. This would suggest that an infant's dietary exposures influences their growth which in turn influences their neurodevelopment achievements. Although growth is not the only predictor of neurodevelopment outcomes it is important to consider that whilst attempting to control postnatal growth to prevent later obesity, we need to also ensure that brain growth and neurodevelopment outcomes are maintained[90]. It is important, therefore, for us to examine not just the effect of nutrition on growth but also the effect on cognitive development.

In reviewing the current literature on the effects of an infant's milk diet on their body composition, growth and neurodevelopment, most of the available data are from pre-term[91-94] or high risk populations[84-86, 95, 96]. Little information is available on healthy term infants whose milk diet is nearly always parent-led or clinically informed by a healthcare professional. Advice of health care professionals are usually sought when the babies' growth or feeding behaviour leads to parental concern. Clinical trials on the effect of infant formula exposure are few, and mostly industry led. Therefore, we have little information on whether these parent-led choices are appropriate.

It is for these reasons that this thesis has examined the milk diet of term, low-risk infants on their growth, including body composition, and neurodevelopment during the first two years of life.

Chapter 2: Literature Review

A systematic search strategy was used to identify recent relevant empirical literature. This involved searching seven databases using keywords, wildcards, truncations and Boolean operators. Only papers written in English were sourced and this is a limitation of the literature review. A full list of the literature search strategy is provided in Appendix 2.

2.1 Milk diet of infants

Nutrition in early infancy has been proposed as a predictor for later-onset outcomes such as obesity. Therefore, as early nutrition is a predictor for health outcomes it is also viewed as a modifiable intervention to improve health[97]. Prior to examining the effect of early nutrition or even investigating how to modify nutrition in early infancy it first needs to be established what the current guidelines are, what are the current feeding practices and what are the determinants of these identified feeding practices. As milk is nearly always the sole diet of infants in the first few months of life and is the exposure of interest in this thesis the guidelines for breastfeeding and type of infant formula will be discussed. This will be followed by a review of the milk (breast-milk and infant formula) diet of infants in the first 12 months of life from developed countries that have undertaken national infant feeding studies (and published their findings in English), with particularly focus on the habits of Irish families.

2.2.1 Breastfeeding

Since 2001 the WHO recommends that all infants are exclusively breastfed for the first 6 months of life and continue to receive breast-milk up to two years of age and beyond along with complementary food[1, 98]. Most countries have adopted the WHO recommendation for breastfeeding, including Ireland in 2003[2].

Systematic reviews that support the WHO recommendation[1, 98-100] have compared the benefits of exclusive breastfeeding for three to four months against six months. Results from the UK's Millennium cohort found that the risk of gastroenteritis was increased when exclusive breastfeeding was stopped due to the introduction of infant formula but not solid food[101]. The European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) report that there is currently no evidence, in developed countries, to support any potential risks to the breastfed infant when solid food is introduced at four to six months compared to after six months[102, 103]. ESPGHAN reported no potential risks when exclusive breastfeeding is stopped due to the introduction of solid food.

The recommendations of the WHO vary from the recommendations of the European Academy of Allergy and Clinical Immunology (EAACI)[104]. EAACI's advises that exclusive breastfeeding should be undertaken for a period of four to six months and that avoiding the introduction of solid food beyond four months offers no protective benefit in preventing food allergy in infancy. At the 2014 and 2017 annual EAACI conferences it was advised that the benefits of breastfeeding (for food allergy prevention) are only available during the period of breastfeeding itself. Introducing solid food while the mother continues to provide breast-milk may help to prevent food allergies in the infant. However, once breastfeeding stops the protective benefits are no longer available to the infant[105]. This suggests that the available benefits of breastfeeding are not solely dependent on the exclusivity of breastfeeding (as promoted by WHO) but also to the duration of breastfeeding. How exclusive breastfeeding is ceased could just be as important as the duration of exclusive breastfeeding itself.

It is important when appraising the quality of evidence on infant feeding that the same rigour that is applied in examining the composition of breast-milk and breastfeeding guidelines is also applied to the assessment of the composition of infant formula and infant formula guidelines. This ensures that all features of infants' milk diet are evaluated to fully inform the potential benefit and risks of all aspects of their milk diet.

2.2.2 Infant formula

International paediatric[65] and dietetic associations[5, 66] and public health bodies[6, 7], including Ireland[67], recommend a standard whey-based infant formula for the first 12 months of life and then full-fat cow's milk, unless medically indicated (for reasons such as cow's milk allergy or phenylketonuria).This is because breastmilk is whey-based and whey is more easily digestible than casein[64]. Standard whey-based infant formula are also referred to as 'first milk' or 'milk for the newborn baby'.

Infant formula is an expanding business and the number of non-standard whey based infant formula, advertised as suitable from birth, is increasing. In 2013 the global baby food industry estimated value was GB£23 billion (US$36 billion) and most of that worth was driven from infant formula, which was valued at GB£16 billion (US$25 billion). Nestlé SA is the world leader with 23% hold of the marked followed by Danone (14%) and then Mead Johnson (11%)[3]. Infant formula that are available and advertised as suitable from birth include whey or casein based, partially or extensively hydrolysed whey or casein based or soya-based formula.

In view of the expanding infant formula market and the nutritional diversity of breast-milk, ESPGHAN recommend that the composition of infant formula be determined by comparing the physiology (such as growth patterns), biochemical (such as plasma markers) and functional outcomes (such as immune responses) of exclusive breastfed to those of formula fed infants[106]. In reading papers from Nestlé SA[107], Danone[108] and Mead Johnson[109] it is evident that this recommendation has not been met in the development of their infant formula products, however, no explanation for this could be found.

In summary, infant formula is a static fluid which can be whey-based, casein-based, soya-based or lactose free. For infant formula, advertised as suitable from birth, there is little difference in the energy and fat levels between the different infant formula but protein and carbohydrate levels can vary between 1.3-1.8g/100ml and 6.8-7.8g/100ml, respectively. It is important to note that infant formula cannot simulate the nutritional diversity and richness of human breast-milk, which adapts and changes to suit the age, health and development of the infant. Yes, there are different infant formulae available (different nutritional content and taste) but each infant formula in itself is a static fluid. For example, the breastmilk a mother produces for her term infant at one week of age will contain 1.98g of protein per 100ml. By two months this drops to 1.32g/100ml and by six months protein levels have decreased to 1.14g/100ml. In comparison, independent of their age, a formula-fed infant on SMA Gold will receive 1.3g/100mls of protein[110]. Their intake of protein will only change if their infant formula is changed. If that same child was on SMA Stay down then their protein intake is 1.6g/100mls.

With such contrasting nutritional diversity between the two methods (breast-milk or infant formula) of feeding, it is important to investigate how infants are being fed.

2.2.3 Milk diet of infants in the first 12 months of life

The initial diet of all infants is milk. Breast-milk is the optimal diet[1] and infant formula is available when breast-milk (either mother's own or donor breastmilk) is not[51]. The milk feeding practices of infants, breast-milk and infant formula, in Ireland and internationally (Australia, Italy, Norway, United Kingdom and United States of America) will be discussed below. Discussion of international milk feeding practices is limited to developed countries with a national infant feeding survey that was published in English. This is to enable direct comparison of Ireland's milk feeding patterns with other comparable developed countries.

There are two national monitoring systems in Ireland to record infant feeding practices in the first three months of life only. The first is the Public Health Nurses (PHN) notification form which is completed following delivery at the point of discharge from a maternity setting and this data is made publicly available in the annual Perinatal Statistics Report. The Perinatal Statistics Report was published by the Economic and Social Research Institute up to

2014 and is now released by University College Cork (UCC)’s National Perinatal Epidemiology Centre. These reports only capture the feeding practices of infants discharged from an Irish maternity setting but not from the small number of home births and do not capture what method of infant feeding was initiated in the maternity setting. Current definitions[111] of infant feeding include exclusive breastfeeding (not stated if this includes infants who received infant formula supplementation), any breastfeeding (exclusive and combined feeding) and artificial feeding. The data collected from the PHN notification form is used as a performance indictor for health outcomes by the Irish Department of Youth & Child Affairs[112]. The second assessment of infant feeding is completed when the child has a three-month assessment by a PHN. Mothers are asked how they fed their infant in the past 24 hours only and not how their child was fed up to or including three months of age. PHNs record the reported method of feeding at the three months’ assessment into the Health Service Executive (HSE) database. This data is not linked to the first monitoring system (so it cannot track any changes in infant feeding in the first three months of life) and is not publicly available. The number of infants who receive their three-month check-up or the reported rates of infant feeding at three months is not reported as a performance indictor by the Department of Children and Youth Affairs. Therefore, only the method of infant feeding at discharge from the maternity hospital will be discussed in this thesis and not the method of feeding at three months as it is not available. Nearly all Irish data that is gathered on infant feeding focuses on breastfeeding thereby excluding infants who are not receiving breast-milk.

All Irish infant feeding studies[113-115] collected data on the method of infant feeding at discharge from the maternity hospital. Since the first survey in 1981[113] to the last in 2008[115], breastfeeding rates improved by 14.5% over a 27 year period and breastfeeding with infant formula increased by 9.2%. Over the course of a generation exclusive artificial feeding decreased by 24.1%.

The changing trend of infant feeding practices at discharge can be seen more clearly when the data from the annual Perinatal Statistics Reports is examined. Figure 1 shows the data from all Perinatal Statistics Reports for singleton births from 1999 up to and including 2014[111, 116-129].

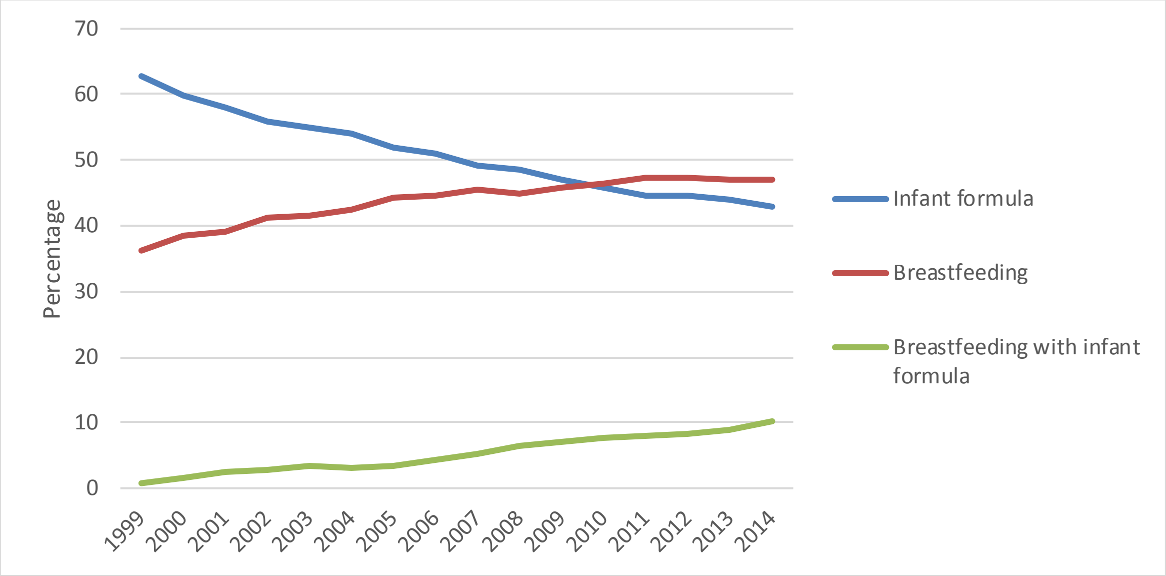


Figure 1: ***Infant feeding on discharge from a maternity setting***[111, 116-129]

As seen above, 2010 was the first year for reported breastfeeding rates to exceed rates of infant formula feeding at discharge and this trend has continued upwards to the most recent report for 2014. The increase in breastfeeding coincides with a change in the maternal population, including maternal country of birth. Brick & Nolan (2013) examined the effect of maternal characterises on breastfeeding rates from the 2004-2010 Perinatal Statistic Reports. The authors reported that maternal country of birth, followed by increasing maternal age at delivery, had the strongest effect on breastfeeding rates at discharge[130]. This is suggesting that rates of breastfeeding are improving due to increasing numbers of non-Irish mothers and rates of breastfeeding among Irish mothers are remaining relatively unchanged[131]. Infants born to Irish mothers are not experiencing the same increase in breastfeeding rates compared to infants born to immigrant mothers.

Records of infant feeding following discharge from the maternity hospital mainly focused on breastfeeding practices and only the 1981[113] survey collected data on infant formula feeding practices. This 1981 survey reported that by six weeks postpartum 34% of mothers had changed their infant's formula, primarily because they felt that the current formula didn't suit the baby (wind, constipation, vomiting or hungry baby).

The last Irish infant feeding survey, in 2008[115], reported that by three months rates of exclusive breastfeeding, partial breastfeeding and infant formula feeding were 19%, 15% and 66%, respectively. By six to seven months' post-delivery exclusive and partially breastfeeding rates had dropped to 13.3% and 4.4%, respectively and the majority of infants (79.26%) were now having some form of solid food in their diet.

The first longitudinal, but not national, study of infant feeding was carried out as part of a Eurogrowth Study which involved 21 centres and about 2,000 infants. The Irish arm of the study was undertaken by Trinity College, Dublin, and recruited 121 infants at birth in 1992. Results from the Eurogrowth Study[132] showed that Ireland had the lowest rates (26%) of any breastfeeding at 1 month of age across all 21 European study sites. By 12 months only six infants were receiving any breast-milk. The majority of infants were receiving infant formula by one month of age. The authors reported that most infants started on a whey-based infant formula and gradually changed to casein-based by nine months of age. Follow-on infant formula was only recently introduced to the Irish market at the time of study and consumption of follow-on formula was at its highest at nine months (n=11, 13.41%). After nine months parents tended to give their infants shop bought cow's milk.

The Growing Up In Ireland (GUI) is Ireland's first national cohort study of infants from nine months of age (arm one)[133] and children from the age of nine years (arm two)[134]. The infant cohort recruited 11,100 infants, out of a total population of 41,185 infants, who had registered for child benefit in 2008 (born between December 2007 – May 2008)[135].

The GUI infant cohort study reported that 49% of the infants were receiving breast-milk (either exclusive or with infant formula) on discharge from the maternity hospital. The mean duration of breastfeeding was three months (11 weeks for Irish-born mothers and 14 weeks for non-Irish born mothers). The study records a rate of 2% exclusive breastfeeding (no infant formula or introduction of solid food) at nine months, with 9% still being partially breast-fed. No other information is provided on rates of exclusive breastfeeding at other time-points prior to nine months of age.

Overall, Ireland has repeatedly shown low breastfeeding rates and has paid little attention to the feeding practices of non-breastfed infants in the first year of life. Maternal characteristics found to be consistently associated with rates of breastfeeding were: maternal age, employment status and education. Infant or paternal characteristics have not been investigated.

To allow comparison of the milk feeding patterns of Irish infants to other countries, I will next present data on national infant feeding practices in the first year of life from Australia[136], Italy[137], Norway[138, 139], UK[140] and United States of America (USA)[141-143]. Duration of data collection varied from the first six months of life[228] up to the infant's first birthday[136, 137];229;[141-143]. The dates of when the infant surveys were completed ranged from 1998 (Norway)[228] to 2011 (Australia)[136]. The UK has carried out an Infant Feeding Survey (IFS) every five years from 1975 up to and including 2010. In this thesis the results from the final survey (2010) are discussed.

Rates of exclusive breastfeeding at six months of age varied from <1% (in the UK)[140] to 10% (in Norway)[229]. All national infant feeding surveys reported on breastfeeding patterns and practices but only two, from UK[140] and USA[141-143], provided information on infant formula feeding practices. Results from the UK's IFS showed that by four to six months after delivery most (88%) mothers were availing of infant formula and 9% had already started to give follow-on formula. By eight to ten months postpartum 35% of mothers were still using infant formula and 57% follow-on formula. The Infant Feeding Practice Study II (IFPS II) from USA also collected information on type and changes of infant formula but did not group infant formula by their protein composition. The IFPS grouped infant formula as: amino acid based, milk based without docosahexaenoic acid (DHA) & arachidonic acid (ARA), milk based with DHA & ARA, soy based with DHA & ARA, soy based without DHA & ARA and extensively hydrolysed casein hydrolysate with or without DHA & ARA. The majority of infant formula changes were switching from a DHA and ARA based infant formula to another type of formula[142]. The authors reported that formula changes were made for mainly non-health reasons (a health reason was defined as that based on stool characteristic or diarrhoea, vomiting and fussiness)[143].

Infant feeding encompasses all forms of feeding that the infant receives and not just breast-milk. The health of all infants matters and if we do not monitor the feeding practices of infants who are not breastfed, the impact of their diet cannot be evaluated. It currently remains unknown if the parents of formula-fed infants are adhering to international recommendations on the type of infant formula which they provide to their infants.

The data from the Norwegian infant feeding surveys show that Norway's image of a country with high breastfeeding rates comes from any and not exclusive breastfeeding in the first six months. Norway has higher exclusive breastfeeding rates compared to many other developed countries (such as Ireland, United States of America, United Kingdom) but even Norway does not have a high exclusive breastfeeding rate at 6 months (10% in 2008) as recommended by the WHO[144].

Similar methodological difficulties and variation in definitions are observed in most of the national infant feeding surveys. The Australian[136] and Italian[137] infant feeding surveys do not provide definitions of breastfeeding in their analysis and results. Significant variations in the definition of breastfeeding are present in many of the reported infant feeding studies[145].

Another significant challenge to breastfeeding surveys is the difficulty in controlling or reducing recall and responder biases[146-154]. Possible reasons for the misclassification of the duration of exclusive breastfeeding could be the interviewing technique of the interviewer and how the parent interpreted the questions. Asking a mother how long she breastfed for exclusively is different from asking her how old her infant was when s/he first had complementary foods[151, 152]. Research has shown mothers may not consider a temporary change in how their infant was fed as the point that they stopped exclusively breastfeeding. Studies have reported that shifts in type of breastfeeding (i.e. exclusive to predominate) are not uncommon and therefore mothers may not count these temporary changes in breastfeeding behaviour when reporting on duration of exclusive breastfeeding[152, 153].

The structure of the questions used on method of feeding by the various national infant feeding surveys also influenced the rate of breastfeeding reported. Australia[136] and America[141-143] collected the method of infant feeding at a specific time period, whereas Norway[138, 139] retrospectively collected their data. Both these methods can result in exclusive breastfeeding being over-estimated and infant formula feeding under-estimated.

The proportion of available infants who participated in national infant feeding surveys ranged from less than 1% (USA– IFPS II)[141-143] to 58.6% (Norway's 1996 infant feeding survey)228. The infant feeding surveys from the UK[140] and Australia[136] weighed their selection to account for responder bias. Italy[137] and USA[141-143] who reported the lowest participation rate, in comparison to the national birth rate, were unable to correct statistically for their small sample size.

All health research has the potential to impact on public health interventions and guidelines. For objective guidelines to be developed all areas of an infant's milk diet need to be examined. The national surveys had little data on the number of parents who changed infant formula, the use of follow on formula and compliance with international recommendations on type of protein content. Only one national survey (IFPS-II from USA) examined infant formula changes and only the UK's IFS reported the proportion of infants that changed to follow-on formula.

2.2.4 Studies examining parents changing their infant's formula

Studies examining parents changing their infant's formula were conducted in USA[155, 156], UK[157], Israel[158], France[159] and Ireland[160]. The only studies to report on the type of infant formula based on protein composition are from UK and Ireland[160]. As the findings from both the UK's IFS and USA's IFPS II have already been discussed they will not be re-reviewed here.

The American studies which focused on infant formula change were conducted in Connecticut (1980)[155] and Michigan (1995 & 1996)[156]. Both studies examined changing from a 'standard' to a 'nonstandard/special' formula in the first four and seven months of life, respectively. The studies showed that 21.6% of infants in the first four months of life[155] and 36.5% (58/159) of infants (age range 30-210 days)[156] had their infant formula changed at least once. The main parental reasons reported for changing infant formula was crying[155] and spitting up[156].

The UK study[157], from Sheffield, reported on the type of infant formula (whey- or casein-based) use in the first six weeks of life for term, singleton infants in 1989. The data collected was from a prospective preliminary survey to a RCT and reported that by six weeks of age 30/173 (17.3%) infants had their whey-based formula changed to a casein-based formula (reasons not listed). At the point of this survey the recommendation, in the UK, was that formula-fed infants should be given a whey-based infant formula.

The next study to report on type of infant formula use was undertaken in Israel between 2002-2003[158].No information is given as to how old the infants were when their parents completed the questionnaire, maternal parity, gestational age at delivery or any paternal information. Besides maternal education no other SES information is given. The authors state that 47% of infants experienced at least one infant formula change, but it is not clear if that is 47% of all infants (n=200) or 47% of infants who received infant formula (n=169). Most changes (67%) were to another cow's milk infant formula but unfortunately the authors do not define the infant formula as whey-based or not. On average, most changes occurred in the third month of life; first change at mean(SD) 3(1.7) months and second change at 3.5(1.7) months.

The most reported reason for switching infant formula was regurgitation/ vomiting (24%), followed by the infant being ‘restless’ (18%) and 15% of parents reported no particular reason for switching their child’s infant formula. The remaining reasons listed were: ‘baby objection’ (13%), diarrhoea/ constipation (13%), advertisement (8%), doctor recommendation (4%), rash (5%) and cost (3%). The only demographic factor found to influence formula changes was the mother’s education level. There was an inverse relationship between amount of years in education and number of infant formula changes.

The Étude des Déterminants pré et postnatals du développementet de la santé de l’ENfant (EDEN) cohort in France also examined infant formula changes[159]. The aim was to investigate the parental, infant and healthcare predictors of type of infant formula and examine the effect of the predominant type (regular, partially hydrolysed, thickened (but not with pre-/probiotics) and infant formula enriched with pre-/probiotics) of infant formula in the first four months of life on growth. Sixty-one percent of infants experienced a change in their infant formula and out of those 26% had two or more changes. No one paternal or infant characteristic was associated with type of infant formula provided. The study found that there was no link between the type of infant formula used the most (predominate infant formula) and growth in the first four months of life.

The final paper, from Ireland, descriptively reported on type of infant formula given to infants six weeks after delivery[160]. The study reported that out of the 368 formula-fed infants 53.6% (n=197) were being given a standard whey-based infant formula and 49.2% (n=181/368) had had experienced at least one formula change. For infants whose formula was changed, either to a whey-based or non whey-based infant formula, parental reports of their infant’s increased hunger/ feeding frequency of 2-3 hours was the most (n=108/197, 54.8%) reported reason. The study did not provide any information on the initial infant formula. It is not known if the infant formula that was changed was whey-based or non whey-based, what parental characteristics were associated with the type of infant formula given to the infants or what type of infant formula was given after six weeks of age.

Overall, no national infant survey examined if formula-fed infants received whey-based (international type of recommended type of infant formula) infant formula or not. No one paper examined the associated characteristics with guidelines compliance and only two reported on the type of infant formula at six weeks of age. This is in stark contrast to the numerous research studies that have examined many different facets of breastfeeding.

Diet (breastmilk and/or infant formula) is one of the biggest environmental drivers of infant growth in the first few months of life. It is therefore important that when examining an infant’s milk diet that the predictors and patterns of growth are also explored.

2.3 Growth in early childhood

Infant and child growth is closely monitored throughout infancy as an indicator of health and nutritional wellbeing43;[161, 162]. Growth charts are universally used by healthcare professionals to record and monitor growth but how growth is defined, measured and monitored varies. Historically, growth charts described how infants and children should grow, independent of their feeding status (i.e. breastfed or formula-fed, introduced to solid food or not). Overall, most growth charts were developed from formula-feeding populations. However, the WHO’s MGRS and the International Fetal and Newborn Growth Consortium for the 21st Century Project (INTERGROWTH-21st Project) have now created growth charts based on the reference data of infants who were exclusively breastfed for least four months Both these growth charts are based on how infants and children should grow when in an environment (i.e. exclusively breastfed, non-smoking mother) that promotes health and wellbeing.

Both the WHO's MGRS and INTERGROWTH-21st project growth charts are centile growth charts. Centile growth charts show how a child's growth compares to his/her healthy peers. The interpretation of the growth pattern is done by the healthcare professional and allows the healthcare professional to track whether a child is following a normal growth trajectory.

Changes in an infant or child's growth in comparison to their peers or predicted growth trajectory can be described by stating how their weight compared to the population mean has changed over time. One method of simplifying this calculation is by the use of z-score. The width between each major centile line on a growth chart is approximately 0.67SD. Infants that show a change greater than 0.67SD or an increase in z-score of ≥ 0.67 in their original z-score experienced greater growth (increasing by one (or more) major centile) compared to infants that did not experience a change of >0.67SD. Rapid growth is often defined as a z-score change of at least +0.67SD and infants who experience rapid growth[122;155-167; 168-172], particularly in the first two months of life[27-29, 32], are shown to be at later risk of overweight and obesity. Diet is the principal driver of growth during infancy[163] and in the next section instruments used to measure infant growth, predictors of growth and the effects of early rapid growth will be discussed.

2.3.1 Growth of term infants in the first two years of life

Growth is routinely recorded on centile growth charts, which capture previous and current assessments to provide information on not just size, but growth patterns too. Monitoring growth is important for the evaluation of both individual and population level health and nutritional status. Serial assessments provide information on how an infant's diet, environment and/or health impacts on their growth and allows clinicians to evaluate the effectiveness of any interventions implemented to support infant growth.

The two most recent, international, growth reference data-sets for term, singleton infants born to an unspecific/general population come from the WHO's MGRS[37, 164, 165] and the INTERGROWTH-21st Project[38, 166]. The INTERGROWTH-21st Project was set up to complement the MGRS by providing fetal reference growth data. The Newborn Cross-Sectional Study (NCSS) from the INTERGROWTH-21st Project also provided reference birth data for both preterm (≤36^{+6} GW) and term (≥37^{+0} GW) infants in the first eight months of life[38] but as this thesis focus is on full term infants the remainder of this chapter will only focus on the reference data for term infants from the NCSS.

The MGRS and NCSS produced similar anthropometric measurements at birth. The MGRS reported that at birth, infants had a mean(SD) weight, length and head circumference of 3.3(0.5)kg, 49.6(1.9)cm and 34.2(1.3)cm, respectively. Infants who participated in the NCSS had a mean(SD) weight, length and head circumference of 3.3(0.5)kg, 49.3(1.8)cm and 33.9(1.3)cm, at birth, respectively. Both the MGRS and NCSS provide for international standards on the weight, length and head circumference of term infants at birth. The MGRS, using a longitudinal design, extends these standards in to the first two years of life. Both reference datasets allow for international comparisons on neonatal and child growth, which will enhance our knowledge in the field of childhood growth. The WHO Growth Standards for Infants and Young Children from the MGRS have been endorsed by the European Childhood Obesity Group, International Paediatric Association, United Nations Standing Committee on Nutrition and the International Union of Nutrition Sciences[11]. By 2011 the WHO Growth Standards for Infants and Young Children had been adopted by 125 countries and was being considered for adoption by 25 countries[43]. Since 1st January 2013 Ireland has used the UK-WHO Growth standards for use in monitoring childhood size and growth in Ireland[42].

In evaluating the appropriateness of implementing the WHO growth standards on a global scale, a systematic review found that the head circumference from the WHO growth standards showed the greatest geographical variation when compared to local reference data and this was followed by weight and then length[167]. The results from the review would suggest that healthcare professionals should remember the variation in mean scores between height and weight when assessing for failure to thrive. It also suggests that the WHO growth standards may need to be modified to adapt to the local population, as done by United Kingdom (UK)[168]. The UK-WHO Growth standards uses the birth data from the UK 1990 growth reference dataset and then data from the MGRS for infants aged two weeks and older to best reflect neonatal size at birth and childhood size and growth for children in the UK.

2.3.2 Maternal antenatal and initial infant feeding characteristics and growth from birth up to 2 years of age

It is clear that the first two years of life are a time of rapid growth, and that this growth can have far-reaching consequences on life-long growth and health. Many factors may affect the growth of a child during this time, related to the child's early environment. In the following section I will address the most significant exposures which may influence early growth[169-171].

Maternal smoking: One of the factors with a direct and significant effect on fetal growth is maternal smoking. Infants born to mothers who smoke are proportional smaller at birth; with both reduced birth weight and length[172-179]. Studies that have examined the effects of maternal smoking in pregnancy on childhood overweight and obesity after birth have reported inconsistent results[172-174, 179-183]. Overall the majority of studies reported that term singleton infants born to mothers that smoked in pregnancy gained more weight compared to term singleton infants born to non-smoking mothers[174, 179-182]. This may lead these children to catch up with their peers to the point where there is no longer any difference in size between the two groups[174, 179] or that infants born to mothers who smoked were heavier in childhood compared to infants born to non-smoking mothers[180-182].

Gestational Diabetes Mellitus: Studies examining the effect of GDM have shown varying results[93, 184]. A Chinese birth cohort reported infants born to mothers with GDM had high neonatal weight-for-length z-scores, but in the Brazilian cohort[93] infants born to mothers with GDM had the lowest weight-for-length z-score. Fasting plasma glucose levels at 28GW from non-diabetic mothers correlated with weight, length and BMI at birth[185, 186] but not at three[185], six[186], 12[185, 186] or 24[185, 186] months. A German cohort[187] did not find a consistent relationship between BMI group, GDM state and anthropometric measurements throughout the first year of life. Results from the Growing Up in Singapore Towards Healthy Outcomes (GUSTO) Study[188] found that mothers with GDM were older (by a mean two years) and had higher rates of education compared to mothers without GDM. Both groups were classified as overweight but mothers with GDM had a higher BMI compared to mothers without GDM, 27.1 and 25.9, respectively. The authors found that being (any or fully) breastfed or not for either <4 or ≥4 months did not affect the infant's BMI standard deviation score (SDS) at any time in the first 36 months of life if the mother did not experience GDM. For infants born to mothers who did experience GDM being breastfed (any and full) for ≥4 months significantly reduced the BMI SDS at 6 months of age compared to not being breastfed (any or fully) for ≥4 months (0.49, 95% 0.04-0.95, and 0.58, 95% CI 0.16, 0.99, respectively) when controlling for maternal age, ethnicity, education, parity, BMI and gestational age at delivery.

Maternal body composition: Studies have shown a gradient relationship between infant weight at birth and maternal Body Mass Index (BMI)[172, 189, 190]. Infants born to mothers with a normal BMI (18.5-24.9 kg/m^2) gave birth to infants who weighed less compared to overweight (25.0-29.9 kg/m^2) or obese (>30.0 kg/m^2) mothers[191-193]. These studies did not show a difference in early weight gain between infants born to mothers with a normal, overweight or obese BMI.

The reported effect of maternal BMI on infant length is inconsistent[172],[190]. Results showed that infants born to underweight, overweight and obese mothers grow at a slower rate compared to infants born to normal weight mothers[172]. However, other findings have shown that all infants, regardless of maternal BMI group, grow in length at the same rate in the first year of life[190].

Gestational weight gain (GWG)[172] has a significant effect on weight and length size at birth and at three months but not on weight or length gains. When examining GWG based on maternal BMI group[190], as per the recommendations of the Institute of Medicine (IOM)[194], being born to an underweight mother, regardless of her GWG category, was protective, for her infant, against overweight and obesity at 12 months of age. Being born to a mother with a normal, overweight or obese BMI who had excessive GWG increased the odds of being overweight or obese at 12 months. Infants born to mothers with a normal, overweight and obese BMI who gained the recommended GWG did not have a significant increase in being overweight or obese at 12 months[190].

Overall, the varied results of these studies[93, 172, 184-187, 189, 190, 195, 196] highlight the difficulty in elucidating the mechanisms through which metabolic stress (BMI, GDM and GWG) affects infant growth. Potential reasons for the inconsistent results include the fact that not all variables were described[189, 190], maternal and infant anthropometric measurements were self-reported or estimated using regression based on other measurements[172, 187, 189], small sample size[187] and the different covariates collected by each of the studies, making direct comparison difficult.

To-date studies have shown that GWG and not BMI influence infant gain after birth[172, 190] and excessive GWG, independent of BMI, has been shown to increase the risk of developing GDM[197]. This suggest that maternal GWG is the best predictor to modify in order to optimise infant weight and not maternal pre-pregnancy BMI[172],[190]. These results also suggest that maternal glucose levels, and not maternal pre-pregnancy BMI, predict infant size or modify how GDM affects infant size[185, 186].

Hormonal appetite regulation: Infant dietary intake is also driven by appetite. Appetite regulation is, in part regulated by hormones secreted from adipose tissue, termed adipokines. One of the most studied adipokines in the field of obesity research is Leptin. Leptin, has been implicated in the regulation of growth through appetite regulation. Leptin regulates nutritional intake and growth through its anorexigenic effect by promoting satiety. As a result of how leptin modulates appetite, leptin is considered one of the most important hormones related to adipose deposition[198].

How much leptin is produced by the body is related to the body's fat mass but it is important to note that interperson variability can influence leptin levels[199]. One important factor which influences leptin levels is gender. Female infants have significantly higher leptin levels at birth compared to male infants[199-202] and one of the suggested reasons for this is that female infants have a higher percentage fat mass to male infants. It is proposed that infant's with a low fat mass will also have lower levels of leptin, compared to infant's with a higher fat mass[203]. As a result of the low levels of lipid infant growth is promoted due to the decreased satiety.

Leptin is one of the biologically active factors, found in breastmilk, which although isn't a nutrient has a role in regulating the metabolic pathways[204, 205]. Leptin is not present in infant formula. As leptin mediates both the metabolic and endocrine system this could be one of the reasons why there are differences in body composition between breastfed and infant formula fed infants[199, 206].

Type of milk feed: The WHO recommend exclusive breastfeeding for the first six months of life[207] but Cochrane reviews examining the benefits of exclusive breastfeeding for three-four months versus six months have not found an effect on weight-for-length z-score at six months ($p=0.34$), nine months ($p=0.49$) or 12 months ($p=0.07$)[208]. Recent meta-analyses[10, 209], examining breastfeeding compared to not breastfeeding on obesity, have reported similar pooled odds ratio: OR 0.74 (95% CI 0.70-0.78)[209] and OR 0.78 (95% CI 0.74-0.81)[10]. Both meta-analyses reported that exclusive breastfeeding was more effective than any breastfeeding in combating obesity but the strength of effect differed, OR 0.69 (95% CI 0.61-0.79) and OR 0.80 (95%CI 0.71-0.90). One of the meta-analysis also showed that how exclusive breastfeeding, mixed feeding and exclusive infant formula feeding were defined altered the protective effect of breastfeeding, compared to formula-feeding, on later obesity risk[10]. The meta-analysis from Hotra et al (2015)[209] found that the longer the follow-up period the less protective breastfeeding was against obesity: 1-9 years of age OR 0.74 (95% CI 0.68-0.79), 10-19 years of age OR 0.63 (0.54-0.73) and ≥ 20 years of age OR 0.88 (95% CI 0.82-0.94). This indicates that other factors and exposures gain in importance over time.

Few studies have examined the effect of an infant’s milk diet at 2 months of age on their growth in the first two years of life. Researchers from the Bassett Mothers Health Project (New York, America) examined the effects of any breastfeeding of less than two months, two to four months and greater than four months on weight gain in the first two years of life[210]. They reported that there is some evidence that breastfeeding may negate or abrogate other risk factors. Infants who were at high risk of increased weight gain (had at least two risk factors) and were breastfed for less than two months had over two times the odds of having rising weight gain trajectory (OR 2.55, 95% CI 1.14-5.72, p=0.02) compared to infants with at least two risk factors for increased weight gain but were breastfed for greater than four months. Limitations of this study include that no information was given concerning other aspects (i.e infant formula, age at introduction of solid food) of infant diet. Infants were measured at no pre-defined ages; all assessments were extracted from routine healthcare checks. Maternal but no infant characteristics are given by the study, with the exception of child PI.

Data from LISA (Influences of life-style factors on the immune system and the development of allergies in childhood) study found that the rate of excessive weight gain (z-score change greater than 0) decreased in infants with longer exposure to exclusive breastfeeding (14.7% for infants classified as exclusively breastfeed from birth to one month compared to 6.7% for infants exclusively breastfed for at least six months)[211]. GECKO Drenthe Birth Cohort[170] [212] found a significant interaction between type of milk diet (breastfeeding, mixed feeding and formula feeding) and infant sex on changes seen in WFL z-scores. Table 1 shows that male breastfed infants experienced greater WFL z-score changes compared to female breastfed infants in the first six months of life. Table 1 also highlights that male infants, but not female infants, who were formula-fed differed to their breastfeeding and mixed feeding peers between birth and six months.

Table 1: Adjusted mean difference in WFL z-scores from GECKO Drenthe Birth Cohort

	Mean WFL z-scores change (95% CI)	
	Male infants	Female infants
Breastfed		
Birth to six months	0.61 (0.33 to 0.90)^{1,2}	0.09 (-0.19 to 0.36)
Six to 12 months	-0.70 (-1.01 to -0.40)^{2}	-0.32 (-0.60 to -0.05)
Mixed Feeding		
Birth to six months	-0.02 (-0.17 to 0.14)^{2}	-0.14 (-0.30 to 0.02)
Six to 12 months	-0.29 (-0.46 to -0.13)	-0.06 (-0.23 to 0.10)
Formula-Feeding		
Birth to six months	-0.28 (-0.51 to -0.05)	-0.11 (-0.34 to 0.13)
Six to 12 months	-0.18 (-0.42 to 0.06)	-0.11 (-0.35 to 0.13)

1) Difference between male and female infants (p=<0.05)

2) Difference when compared to formula-fed infants (p=<0.05)

It is yet to be agreed in the literature what does drive the sex difference in growth. One of the potential reasons for the growth difference between the male and female infants is that sex hormones contribute to the regulation of energy balance[213]. Another proposed reason is that females have higher concentrations of the insulin-like growth factor (IGF-1) axis compared to males. However, when Closa-Monasterolo et al (2011)[59] explored this theory they found that although the female IGF-1 axis had a stronger response to their diet compared to male infants it did not have an effect on the growth difference.

As highlight by Linda S. Adair there are many methodological issues that need to be considered by examining the results from studies examining an infant's milk diet on obesity[214]. Professor Adair grouped these methodological issues into three main categories: (1) Recall bias and poor characterization of feeding exposures; (2) selection bias and inadequate control for confounding and (3) alternate study designs. Another point to add would be the characterization of the outcomes (time point and definition used). Children can be grouped as either overweight or obese using various definitions. Examples of this include: z-score and percentile classifications from different growth charts; then if the z-score is weight-for-age or weight-for-height or BMI classifications.

One of the possible mechanisms that could partially explain the differences in growth between breast- and formula-fed infants is how they receive the milk (i.e. directly from the breast or via a bottle)[215]. Findings from the (American) Infant Feeding Practices Study II have shown that mode of feeding (and not just type of milk) also influences weight gain in infancy[216]. When exclusively breastfed infants were grouped by mode of feeding (directly from the breast and/or expressed milk from the bottle) a positive relationship was seen between increasing number of bottle delivered feeds and weight gain. Breastfed infants who were fed breastmilk from a bottle and formula-fed infants gained 71g and 89g, respectively, more weight per month compared to breastfed infants fed from the breast. These findings suggest that practices such as encouraging bottle emptying and passive feeding from a bottle versus active suckle from the breast may be encouraging infants to drink more milk than is nutritional required to support their growth. The additional energy intake results in the increased weight gain.

The Greenlight Study[215, 217] examined bottle size and volume of infant formula feeds at two months of age. Parental questionnaires, primarily on infant feeding behaviours and type of milk given to infants, were completed when the infants were, on average, 9.3 weeks old. Only the 378 exclusively formula-fed infants were included in the analysis and bottle sizes were classified as small (<6oz) or large (≥6ozs). Ethnic differences were noted between the groups based on bottle size but no differences were found based on WFL z-score at birth or household income. Overall, infants whose parents used a 'large' bottle had a significantly higher intake compared to those infants given a 'small' bottle, 34.2oz verses 29.7oz, p=0.03, respectively. At the time of assessment, infants given a 'large' bottle had higher mean(SD) WFL z-scores compared to infants who were given a 'small' bottle, 0.36(1.2) versus 0.19(1.1), respectively, although this difference was not statistically significant. By six months the difference in WFL z-score, between both groups was significantly different, 0.44(1.0) in the 'large bottle' group compared to 0.11(1.05) in the 'small bottle' group. Results were not stratified by infant sex and this is a limitation of the study. Just over a quarter (n=81/386, 20.9%) of female infants were given a 'large' bottle compared to male infants (n=90/386, 23.3%) however the study population, overall, had more female infants compared to male infants (52.8% versus 47.2%, respectively). As feeding behaviours differ based on infant sex and infant feeding behaviours are linked to infant growth a repeat of this analysis, stratifying for infant sex, is warranted. It is also not reported if the feeding frequency differed based on bottle size (therefore the amount consumed in a 24-hour period may be the same). Overall, the findings suggest female infants are more likely to be formula-fed but given smaller amounts of infant formula compared to male infants. Using a bottle that contains six or more ounces at two months of age results in greater weight, WAZ and WLZ changes compared to using a bottle that can contain less than six ounces.

Overall, the effect of breastfeeding on infant growth[170, 210, 211, 218] suggests that the milk diet patterns may directly affect infant growth or may modulate the effect of other maternal characteristics on infant growth. Whether this is due to the nutritional, or hormonal constituents of the milk, the rate and mode of delivery of the milk feed is unclear. It would also appear that the effect is influenced by their gender130[212].

To conclude, data examining the effect of various antenatal factors (such as maternal smoking, BMI and GWG) and early infant feeding on growth in the first two years of life is incomplete, with most studies focusing on weight. Papers that reported on length[172-174, 177, 179, 219, 220] and head circumference[173, 174, 179, 220] at birth are limited to those investigating the effects of maternal smoking in pregnancy or umbilical leptin levels.

2.3.3 Early Growth

In 2004 Professors Singhal and Lucas, from the Institute of Child Health in London, published a commentary in The Lancet proposing how the adverse effects of rapid growth in infancy was a risk factor for cardiovascular disease (CVD) and titled their hypothesis the Growth Acceleration Hypothesis[36]. Profs Singhal and Lucas were not the first to explore the effects of rapid growth in infancy but they were the first to link previous research on rapid growth to formulate the Growth Acceleration Hypothesis. They highlighted how historic research, animal studies and clinical trials involving infants demonstrated the long-term effects of childhood growth acceleration on major components of the metabolic syndrome, including obesity. Profs Singhal and Lucas referred to rapid growth as upward centile crossing.

Two years later, in 2006, Drs Ong & Loos from the University of Cambridge, published their meta-analysis examining rapid growth in infancy on later-onset obesity[9]. This wasn't the first meta-analysis to examine rapid growth in infancy but it was the first to provide a method of defining early rapid growth. They converted the reported measures of rapid growth, from 15 studies, to ≥0.67SD increase change in the z-score. This measurement was chosen because the width between each centile band on a standard growth chart is 0.67SD and therefore if an infant experiences upward centile crossing their z-score had increased by at least 0.67SD. They found that all 15 studies showed an increased risk of later-onset obesity following rapid growth in infancy. The pooled odds ratio (OR) (involving 35,835 children) examined the effect of rapid growth in the first year of life on obesity at 10 years of age. In the unadjusted pooled OR infants that experienced rapid growth had nearly three times the odds of being obese at 10 years of age (OR 2.76) and in the adjusted analysis the pooled OR was 1.84, when compared to children who had not experienced early rapid growth in infancy.

The Growth Acceleration Hypothesis continues to be supported by studies[182, 218, 221-234] and reviews[9, 16, 22, 23, 235, 236] which have demonstrated a positive relationship between infants growth rate, upward crossing of the centiles on growth charts, and later-onset overweight and obesity. However, the exact mechanism behind this early rapid growth is unclear.

In the early postnatal period one of the principal driving forces of growth is nutrition intake, driven by both dietary exposure and appetite[8]. In the first few months of life the nutritional intake for infants is milk. One of the main components of breastmilk and infant formula is protein. Protein is an essential building block for cell growth and repair. For infants too much protein can lead to obesity and other metabolic disorders and too little can cause neurodevelopmental delay[237] and weight scores which are below the expect norm. It is therefore important that the protein intake equates the protein requirements of the growing infant[238].

During infancy, breast-milk changes from colostrum, to transitional milk, and again to mature milk, thus adapting to the needs of the infant. Breast-milk differs during a feed (fore- and hind-milk), through the course of the day and stages of lactation. The breast-milk differs between a mother who has delivered a term compared to a preterm neonate. There is intra- and inter-maternal variability in all breast-milk[239]. The infant has influence on the volume, content and frequency of milk ingested[240]. In comparison infant formula is a static rigid fluid that can be either whey-based or non whey-based from birth. In Europe the composition of infant formula is governed by the 2006/141/EC Directive on infant formula and follow-on formula[241], which lists the permitted nutritional content and ranges for infant formula.

The protein levels in breast-milk[242] are significantly different to that found in non-prescription infant formula advertised as suitable from birth. In this thesis the protein reference for breast-milk in term infants will come from Saarela et al (2005)[110]. The authors of this paper differentiated the composition of breast-milk between pre-term and term infants, took into account the age of the infant when the breast-milk samples were obtained and did not pool multiple breast-milk samples (from infants of various ages) into one sample.

The protein content of the various infant formula, all advertised as suitable from birth, ranges from 1.3g/100ml to 1.6g/100ml. In comparison, the protein content of breastmilk is 1.98g/100ml in the first month of life and this drops down to 1.14g/100ml by six months[242]. The point where the protein intake of all infant formula exceeds the protein content found in breastmilk occurs in the second month of life. Between two to three months of age the protein levels found in breastmilk drop from 1.32g/100ml to 1.26g/100ml[242]. It is proposed that the higher protein intake present in formula feeds, may contribute to the increased risk of overweight and obesity of formula-fed infants compared to breastfed infants[52]. The effect of an infant's protein intake on their risk for overweight and obesity has been examined by both observational studies[53, 55, 58, 243-247] and randomised control trials (RCTs)[54, 56, 57, 59-62].

Observational studies examined the effect of the protein intake from all dietary sources and all four RCTs examined the effect of early protein intake from infant formula purposely made for the study, rather than commercially available infant formula, on later onset overweight and obesity. All RCTs had breastfeeding infants as the control group and compared the effect of infant formula with high and low protein levels. The cut off level for high and low protein content was determined by the study authors and did not reflect what is currently available commercially to parents. Results from all four RCTs[54, 56, 57, 59-62] reported a relationship with protein intake and later onset overweight and obesity at 12 months[57], two[56, 62] and six years[60] of age.

As highlighted previously, human breastmilk is whey-based but infant formula can be whey-based or non whey-based (for example: casein, soya-based, lactose-free, partially or extensively hydrolysed (whey or casein)). A meta-analysis published in 2016[248] examined studies which compared the effect of whey-based infant formula (1.8g/100kcal) to breastfed infants on their growth in the first four months of life. It is not clear if the groups were exclusively formula-fed or exclusively breastfed (or if the formula-fed infants were ever breastfed). The authors reported, using WHO growth standards, that breastfed infants, at birth, were significantly bigger in their mean(SD) BMI-for-age z scores compared to their formula-fed peers (0.05(0.99) versus -0.17(1.08)). By four months of age, infants fed whey-based infant formula were significantly bigger and 21% had experienced rapid growth. In comparison, only 9% of breastfed infants saw their weight-for-age z-score increase by ≥0.67SD. The results showed that even using a whey-based infant formula with the lowest, legally permitted, protein levels formula-fed infants experienced more rapid growth to breastfed infants. The authors did not investigate the effect of non whey-based infant formula or if, independent of the protein composition, infant formula use promotes rapid growth.

Another possible mechanism that could explain how protein intake can influence growth is the mammalian target of rapamycin complex 1 pathway (mTORC1). mTORC1 is a nutrient-sensitive kinase and is a key regulator of cell co-ordination, growth and proliferation for muscle, adipose tissue (white and brown fat), pancreas (β-cells and insulin secretion) and liver (IGF-1 production and also contributes to liver regeneration). The mTORC1 signalling system is primarily activated through branched-chain amino acids (principally leucine). The greater the concentration of branched-chain amino acids (BCAA) the greater the signal for the mTORC1 system to increase cell size and growth of adipose tissue[68, 78, 79, 249, 250] [72, 76, 77, 251, 252]. To illustrate this point Table 2 provides information on the milk provided by humans, calves and rats against the desired growth rate of their infants[68]. As illustrated in Table 2, the protein and leucine levels of breastmilk and infant formula vary considerably. The effect of these variations in the levels of protein and leucine levels on early growth, for now, is unclear.

Table 2: Protein composition and content of human, cow and rat milk*[72] *and infant formula and infant growth

Type of milk	**Predominant protein composition**	**Protein content (g/100mL)**	**Leucine content (mg/100mL)**	**Number of days required to double birth weight**
Human (at ~3 months)	Whey	1.2	104	180
Cow	Casein	3.4	333	40
Rat	Casein	8.7	799	4
Infant formula	Whey	1.3	134	Not applicable
Infant formula	Partially whey hydrolysed	1.6	166	Not applicable
Infant formula	Casein	1.6	155	Not applicable

The current available evidence establishes the role of mTORC1 in body weight regulation (energy balance). The activation and regulation of mTORC1 principally comes from milk. All milk is species specific to promote the desired postnatal growth rate of their corresponding infants. As research advances our current knowledge on the mTORC1 pathways and signalling system we will further develop our understanding on what stimulates upregulation of the mTORC1 pathways and how that upregulation results in infant formula feeding -induced obesity.

Overall, there is limited data on the effect of milk diet choice (and the exclusivity of the milk diet) during the first few weeks of life on rapid growth and later onset overweight and obesity. In measuring weight and weight gain it is important to remember that weight is a measure of the body's total relative mass. In investigating weight, the effect of an infant's diet on both their fat and fat free mass needs to be explored.

2.4 Body composition

In the simplest form of body composition analysis the body weight, or mass, is divided into two main components; fat mass (FM) and fat-free mass (FFM). FM refers to the amount of adipose tissue and FFM consists of all non-fat components (i.e. muscle, bone and water) of the human body. The amount of detail obtained from a body composition measurement will be dependent on the method used, i.e. a two-component model measures FM and FFM, and three or greater compartment models measure FM and divides the FFM into its various components (nutritional, metabolic and anatomical models all divide the fat free mass up using a variety of methods)[47].
Examples of two-component models are isotope dilution and air displacement plethysmography (ADP). Isotope dilution is a hydrometric method using a tracer (deuterium or tritium oxide) to determine the total body water (TBW) which gives an estimate of the FFM[44, 45, 253]. In comparison, ADP uses a densitometric technique to directly measure the body volume of the participate[254]. Fat density is considered to be mostly constant[255] which then allows the participant's FM, FFM and %FM to be determined[45].

Multi-component methods include the MRI and DXA. The DXA relies on the attenuation phenomenon of low dose radiation to measure not just FM and FFM but also muscle and bone mineral density for both whole and regional body composition assessments. MRI, in comparison to all other methods discussed, does not measure the body's total fat but the volume of adipose tissue[44] by utilising the Larmor frequency and relaxation time (T1) to produce cross-sectional imagining of the body[253, 256]. MRI can also provide information on the subject's bone structure and muscle and fat distribution.

The precision of any indirect method to accurately report an infant's body composition is reliant on the theoretical model selected to determine the infant's FFM or the formula to calculate their total body fat. Infant reference data, to determine their FFM, are available with both the Fomon[257] and Butte[258] Density Models.

The Butte[258] and Fomon[257] Density models are the two models which are used to determine the density of FFM in a paediatric population. The Fomon Density Model[257] used data from several sources to compile their reference dataset and the methods employed included TBW, total body potassium (TBK), total body calcium and direct chemical analyses was obtained from stillborn infants. The Butte Density Model[258] obtained its data from a longitudinal cohort study, which employed a multi-component method (TBW, TBK and DXA) throughout.

To-date it has been the Fomon Density Model[257] and not the Butte Density Model[258] which has contributed to the current reference body composition datasets for term infants at birth and two months of age. In comparing the two models it should be noted that the International Atomic Energy Agency's report on body composition for children aged 0-2years[259] reported a difference of about 5-7% in the estimated TBW between the Fomon[257] and Butte[258] Density Models for children aged two years or younger**.** Therefore, choice of body composition measurement technique requires consideration of the following:

- Information required: is a two-compartment model sufficient or is more detail regarding distribution of fat free mass required?
- Age of the subject and age appropriateness of the method, for example the PEA POD® is limited to infants less than 8kg, and so not suitable beyond six months of age.
- Acceptability to the patient, for example in young infants MRI or DXA can only take place if they are quiet and still[45, 260]. Between three months and eight years of age it is more likely that children will require sedation, making it less acceptable to parents, and ethically difficult to justify for an observational study.
- Resources (financial and infrastructure related) available to the study. Isotope dilution can be difficult to use in infancy as it is time

consuming, the method requires collection of multiple bio-samples which in turn require resources and the hydration state of infants is not as constant as adults[45].

- Other potential errors in measurement, for example with isotope dilution, errors include stock solution variation and incomplete dosing and or equilibration[259]. In ADP the machine must be regularly calibrated and the room temperature and humidity carefully controlled to ensure robust results.

Currently, MRI has not been validated sufficiently and few studies have examined the accuracy of results generated from the DXA when it is used with infants[259, 261, 262]. MRI relies on the visual interpretation of the scan by the observer, with a formula used to calculate the total body fat. Thus the accuracy of the results obtained from the MRI relies on the skill and experience of the observer making body composition measurement subjective and difficult to compare across studies[193, 263]. Data obtained from DXA varies depending on the scanners or software used, again making it difficult to compare between studies[264, 265]. It is also important to note that DXAs[258, 261, 266] and MRIs[193, 267] have not been widely used in measuring the body composition of children under 2 years of age and concerns have been raised about viewing DXAs as the 'gold standard' in paediatric research at this age[45, 253].

As well as considering the limitations of the various methods it is also important to note their individual advantages over other methods. Isotope dilution is simple to administer and is mobile (which is of particular advantage in field studies)[259]. The PEA POD® is a simple device and takes no more than five minutes to complete the whole assessment and is not reliant on infant behaviour. Studies which have validated data from the PEA POD®[254, 255, 260, 261, 268, 269] have demonstrated both its accuracy and reliability in the body composition measurements of infants. MRI is the preferred method to measure visceral adipose tissue[44, 45] and allows for regional body composition measurements to be estimated. It was through using MRI that researchers found that it is the subcutaneous and not intra-abdominal tissue that is reduced in infants with IUGR[270]. As the DXA provides additional information including bone mass and fat distribution, it is advantageous for studies collecting information on skeletal growth.

Taking these factors into account, the PEA POD® from Life Measurement Inc, Concord, CA was chosen as the instrument of choice for the Cork BASELINE Birth Cohort Study. Overall, the PEA POD®, using the Fomon Density Model, shows good validity and reliability for measuring the body composition of infants in the first two months of life[255, 271]. The PEA POD® is also quick and easy to use and does not rely on infant temperament to complete the assessment. In 2013 The International Atomic Energy Agency published a report recommending the PEA POD® as the instrument of choice to measure body composition in children who weigh between one to eight kilograms[259].

2.4.1 Reference body composition data for term infants at birth and two months

Currently there are four studies which have produced reference data for body composition at birth[272-274];[275]. All but one also included information on the body composition of infants at two months[273, 274] and two and a half months[275]. The PEA POD®, using the Fomon Density Model, was used by all four studies. Two of the studies[273, 274], which provided data at birth and two months of age, replicated the participate criteria from the World Health Organisation's (WHO) Multicentre Growth Reference Study (MGRS). Both of these studies[273, 274] displayed the FM, percentage FM (%FM) and FFM stratified by sex. As illustrated in Table 3 the most striking difference in the reference values, between the two studies, are the female infants. The difference in %FM, for female infants at birth, between the American and Italian cohorts was 4.5%, with American female infants carrying an additional 400g of FM compared to Italian female infants. The observed body weight difference was 60g, so the results from both cohorts would appear to suggest that American female infants are born fatter compared to Italian female infants. By two months the body weight difference had increased by five and a half times to 330g but the difference in FM had reduced down to 150g/ 1.61%. The increased in body weight difference was driven by the changes in FFM. American female infants at two months had an additional 180g of FFM compared to Italian female infants at two months.

In contrast American male infants total body weight was 220g heavier to the Italian cohort at birth but this difference was mainly driven by FFM (140g). By two months the total weight difference had reduced down to 70g and the difference in FFM remained relatively unchanged. Interestingly, at birth it was the American male infants which carried an additional 80g of FM at birth but at two months, this trend reserved, and it was the Italian cohort which carried an additional 80g of FM. This would appear to suggest that as Italian male infants grow, they grow fatter compared to American male infants.

Table 3: Body composition reference values from Roggero et al (2010)[277] ***and Fields et al (2011)***[276]

	Italian Cohort[274]			American Cohort[273]		
	FM (g)	**%FM**	**FFM(g)**	**FM (kg)**	**%FM**	**FFM(kg)**
			Females			
Birth Mean(SD)	260(120)	8.69(3.09)	2710(380)	0.40(0.09)	13.19(2.27)	2.63(0.26)
2 Months Mean(SD)	1090(300)	22.42(3.97)	3700(380)	1.24(0.27)	24.03(3.67)	3.88(0.32)
			Males			
Birth Mean(SD)	290(90)	8.94(2.78)	2910(260)	0.37(0.12)	10.66(2.84)	3.05(0.34)
2 Months Mean(SD)	1360(270)	24.69(3.99)	4130(340)	1.28(0.34)	22.70(4.29)	4.28(0.38)

Two possibilities to explain the results are the sample sizes or that both sex and maternal environment affect body composition to a greater extent than body weight. The Italian cohort had 23 female and 17 male infants assessed at birth but at two months 35 female and 19 male infants were seen. For the American cohort 15 female and 20 male infants were seen at birth and 60 female and 68 male infants were assessed at two months. No study examined for differences between the infants who did and did not attend at each point. So, it is unknown if the differences are due to the different sample sizes and characteristics of the infants at each of the assessments. Sex is a known confounder for weight and body composition[276] and there is some evidence to show that ethnicity is also an important predictor of body composition[267, 277]. Perhaps differences (maternal environment) within the same ethnicity groups are as influential as ethnicity itself on body composition.

The third study to provide longitudinal reference data for term (≥37 GW) infants was undertaken in Ethiopia[275]. Infants who weighed less than 1500g or had a congenital malformation were excluded from the study but no restriction was placed on infant diet. This study presented the %FM, stratified by sex, and their data is presented in Table 4. For the Ethiopian population, in comparison to the American and Italian cohort, at two months of age there was little difference (0.8%) in the %FM, between male and female infants. As with the Italian cohort female infants had more %FM at birth and two months compared to their male peers.

Table 4: Body composition reference values from Anderson et al (2013)[278]

Age	**%FM Mean(SD)**
Males	
Birth	7.3(4.4)
2.5 Months	25.0(4.9)
Females	
Birth	7.8(3.7)
2 .5 Months	25.8(5.5)

The largest sample size to generate reference data camefrom the Cork BASELINE Birth Cohort Study[272] and included 750 infants born from 36^{+0} GW with body composition estimation in the first 4 days after birth. The Cork

BASELINE Birth Cohort Study presented weight and %FM at birth, stratified by sex and gestational age at delivery. Significant differences in birth weight and %FM were observed between all three groups, a gradient increase in birth weight and %FM corresponded to an increase in gestational age at delivery. The reference data were used to construct a centile chart for %FM, which was not done by any of the previous studies.

Overall, there is limited data on the body composition of infants and this limits the discussion on accuracy of available reference data. Difference in the reference values of %FM between all four studies was noticeable. This may be due to variability in recruitment criteria between studies, and population nutritional or smoking habits, or may be related to the effect of ethnic diversity. It does raise the possibility that perhaps global reference datasets for body composition are not suitable and local body composition growth charts may be required[278].

In investigating infant body composition, it is important to consider what can influence the FM and FFM of infants. In the next section I will explore what factors may determine how an infant gains their FM and FFM.

2.4.2 Predictors of body composition of term infants at birth and two months

Dietary intake has been shown to have a crucial influence in the body composition of preterm (≤ 36^{+6} GW) infants. Results have also demonstrated that length of parenteral nutrition[279] and nutrient-enriched formula[280-283] influence FFM. For term infants the research investigating the effects of infant feeding on body composition at two months of age is limited to two studies[284, 285] and the results are inconsistent.

The first study that examined infant feeding used a study specific, and not commercially available, infant formula and reported on weight, lean and fat mass gains in early infancy[284]. Thirteen exclusively breastfed and fourteen formula-fed infants were assessed at a mean(SD) age of 3(1) and 69(5) days, respectively. Definition of exclusively breastfeeding is not given and it is also not stated if the formula-fed infants were ever breastfed, still receiving breastmilk and what age they started the study formula. The authors stated that the reported differences in weight, lean and fat mass gains, between the two-time points, were not statistical significant between the two groups. However, the number of infants studied was very small, meaning that the study was potential underpowered to detect a difference between the groups.

The second study compared the effects of exclusive breastfeeding to mixed feeding on infant body composition at two, four, eight and 12 weeks[285]. All exclusively breastfed infants were listed as receiving no infant formula and for infants categorised as mixed feeders 10% to 100% of their intake was infant formula during the study period. It is reported that breastfed infants had significantly higher FMI and lower FFMI compared to infants on a mixed feed diet. Once weight and length at birth and infant sex were controlled for these differences were no longer statistically significant but the authors do not state what the FMI and FFMI, from their multivariate analysis, was.

It is difficult to directly compare these two studies, as their study populations differed in their method of feeding (exclusively formula fed versus mixed feeding) and how body composition was measured (Pea Pod and DXA). The paper from de Curtis et al (2001) provided limited information concerning the baseline characteristics of their study population which made it hard to examine for differences between the two study populations. In reviewing Anderson's (2009) paper all body composition data is provided as graphs and no body composition measurement is given as written text making it very difficult to assess what the actual body composition measurements were and what differences in body composition exist between the two groups.

Other potential predictors of infant body composition in the first two months of life for healthy, term, singleton infants that have also been examined include umbilical leptin levels[219], maternal smoking[176, 177], maternal dietary intake during pregnancy[286, 287], maternal BMI and weight gain during pregnancy[176, 191-193, 288-291], maternal biochemistry[290, 292-295], maternal physical activity during pregnancy[176, 296], gestational diabetes mellitus (GDM)[178, 297-301] and maternal ethnicity[267, 277].

Variable methodologies and methods were used in these studies. Nineteen studies used the PEA POD®[178, 191, 219, 277, 285, 286, 288-290, 295, 297, 299, 300, 302-306], but the majority did not state what density model they used[178, 219, 277, 285, 288-290, 295, 297, 299, 302, 305, 306]. The remaining studies all reported using the Fomon Density Model[191, 286, 300, 303, 304]. Six studies used the DXA[192, 284, 293, 301, 307, 308] (but limited information was provided on what software was employed[309]) and three studies used MRI[193, 267, 310].

In general the results from these studies indicated that maternal smoking in pregnancy reduced neonatal body mass, with a greater proportion of the reduction seen in the FFM, compared to FM[176, 177]. Dietary studies found that the maternal intake of n-6:n-3 LCPUFA ratio[286], saturated fat[287] and having a high-fat diet increased neonatal %FM[306]. For mothers at risk of developing gestational diabetes their diet (low GI or high fibre) did not affect maternal HbA1c, glucose or HOMA-IR levels or neonatal FM[305]. Maternal leptin levels, taken within 72hrs of delivery, did not show an association with neonatal FM[295]. However, umbilical leptin levels were found to have positive correlation with fat mass at birth and an inverse relationship with FM gain in the first two months of life[219]. Physical activity during pregnancy was not found to have an overall influence on neonatal body composition[176, 296] . However it may not be physical activity alone, but the intensity of that activity which influences neonatal FM[308].

All studies examining GWG found that mothers who gained excessive weight during pregnancy, as defined by the Institute of Medicine (IOM)[194], gave birth to infants with a higher %FM, compared to the infants born to mothers who gained the recommended amount of weight for their BMI[191, 288, 291]. No effect was seen on the FFM. Conflicting results are seen between studies that examined the effect of maternal pre-pregnancy BMI on neonatal body composition[191-193, 290] . Variation in maternal glucose levels during pregnancy may be a confounding factor, and may explain these inconsistencies[191, 192],[290],[292],[178, 297, 299, 300, 310]. Mothers with increased BMI will also frequently have increased fasting glucose levels. There is also some evidence to show that increased maternal HOMA-IR, but not glucose values at 32 gestational weeks' have a positive effect on female neonatal FM but not male FM[304]. This too could further explain the variability of results from studies examining the effect of maternal BMI on neonatal body composition – as not all studies were able to control for neonatal sex.

Study findings also suggest that body composition measurements and changes are, in part, also influenced by maternal ethnicity[273, 274, 303]. Results show that differences based on maternal ethnicity appear at birth and are mediated by neonatal sex[303]. African-American females and Hispanic males showed the greatest %FM at birth, 15.4% and 14.51%, respectively. In comparison, Asian females and African-American males had the lowest %FM at birth, 11.72% and 11.61%, respectively. Findings also suggest that South-Asian infants have a faster growth rate to white European infants in the initial months of life77 and that this increased growth rate is driven by gains in adiposity[267].

As stated previously, evidence on the effects of infant feeding on body composition at two months of age was limited to two studies and the results were inconsistent[284],[285]. What was interesting to note from the available literature was the effect of infant feeding on body composition at birth, prior to the infants becoming 'breastfed' or 'formula-fed' infants[284],[311]. No previous study has explored whether the body composition of breastfed and formula-fed infants differs at birth, prior to them ever receiving their very first feed.

As body composition data becomes increasingly available, our knowledge and understanding of the determinants of early body composition is growing. The PEA POD® in particular has improved our ability to study large cohorts of children and has produced reference data, stratified by age and sex, to allow for both local and international comparisons of other reported body composition assessments. Factors shown to effect infant body composition are maternal smoking, GWG and ethnicity. Further study is needed to examine the effect of infant feeding on infant body composition and if the differences seen in infant body composition, based on method of feeding, existed prior to the infant receiving their first feed, due to associated fetal exposures.

Thus far, this literature review has focused on body composition and growth during the first two years of life. Attention, during this critical period of development, should be more inclusive and not just focus on one area of health. Therefore, the next section will explore the neurodevelopmental skills of children at two years of age.

2.5 Neurodevelopment

The European Union defines neurodevelopment disorders as a functional disability of the brain which affects a child's behaviour and/or ability to learn[312]. Neurodevelopment delay/disorders can affect one domain of development or multiple domains of development. The developmental domains can be categorised into four groups (motor, language, cognitive and personal/social skills). Within these categorised groups the skill sets can be further divided into fine and gross motor skills or receptive and expressive language skills.

Neurodevelopment skills can be evaluated through developmental screening. Developmental screening tools can focus on a specific area or be performed as a general assessment of a child's overall developmental abilities. Many different tools exist to measure a child's neurodevelopment abilities and in selecting which tool to use many considerations need to be undertaken. These include if the questionnaire can be completed by a health care professional and/or legal guardian of the child; desired sensitivity and specificity of the test and area of development to be screened[313-315].

Given that the rate of neurodevelopment between birth to two years of age is unsurpassed at any other time point, attention has been drawn to investigating what stimuli promote or hinder neurodevelopment during this period. Under-nutrition has been shown to negatively affect neurodevelopment but the findings are inconclusive on the effects of over-nutrition[84, 90]. One of the possible mechanisms through which nutrition affects neurodevelopment is its influence on brain growth, as estimated by measurement of head circumference[90].

For large scale birth cohorts, such as the BASELINE birth cohort, formal developmental assessment for all children was too time consuming and therefore prohibitively expensive. Our cohort focused instead on parental questionnaires to estimate development at two years. There are a number of parental questionnaires validated at this age. In the following section I will outline the factors used in choosing a parental questionnaire-based screening tool.

2.5.1 Parental questionnaires to assess their child’s neurodevelopment skills at 2 years of age

A child’s neurodevelopment skills may be evaluated either directly by health care professionals or using validated tools designed for completion by the child’s primary carer. Parent-completed questionnaires are generally preferred for universal population screening, as it saves on resources and is generally acceptable to parents. The Ages and Stages Questionnaire (ASQ), Child Development Inventory (CDI) and Parents’ Evaluation of Developmental Status (PEDS) are validated parental questionnaires which can be used to screen a child’s neurodevelopment skills at two years of age.

It is important to note that the discussion in this section is evaluating how to assess a child’s neurodevelopment. The tools mentioned will assess a child’s ability to undertake certain functions such as reading, memory or social skills. Neurodevelopment screening tools are not an assessment of a child’s intelligence.

The ASQ, which assesses motor (fine and gross), language, problem solving and personal social skills, is currently in its third edition (ASQ-3)[316].The questionnaire is written at a fourth to sixth grade (converts to roughly nine to 12 years of age) reading level. Results given by the developers of the ASQ-3 in the user manual[317] report that overall the ASQ-3 showed strong re-test reliability but the interobserver reliability between trained testers and parents was moderate. Specifically, the 24-month ASQ-3 questionnaire showed strong sensitivity, and this was reflected in their false negative results, but the questionnaire over-identified 13.6% of the target population and their specificity was lower to the combined specificity for all ASQ-3s. Overall, studies have shown that parents find the questionnaire easy and quick to complete[318] and the ASQ has shown strong reliability and validity in a number of studies.

The CDI is designed to assess the motor, language, cognitive and social skills of children from 15 months to six years of age. The CDI can also be used with children who are older than six years of age but have the developmental skills of a child between one to six years of age[319]. The CDI, when compared against the Bayley Scale Infant Development-II (BSID-II), reported 100% specificity for detecting children with a below average score on the BSID-II but the sensitivity value was only 50[320]. Results from other studies would suggest that the CDI can accurately assess which children have a developmental delay but may also over estimate delay and misclassify children with normative function as having a suspected developmental delay. The findings also suggest that the CDI is not as user friendly as the ASQ (parents are more likely to return a completed ASQ compared to a completed CDI)[320-323].

The PEDS questionnaire is designed to assess the four main domains of neurodevelopment for children from birth up to eight years of age. The questionnaire is written at a fourth to sixth grade reading level. Again, it has been found to be easy to complete and acceptable to parents[324]. The reported sensitivity and specificity of the PEDS questionnaire suggest it is more accurate, and therefore more suitable, to use with children who are older than four and a half years of age[325-327].Another consideration to make is that PEDS scores are based on parental concerns and not what their child can and cannot currently achieve. Research has shown that parental concerns is a subjective measurement and is open to different cultural interruption[325].

All three parental-administered questionnaires, ASQ, CID and PEDS have been described by the American Academy of Pediatrics (AAP) as suitable tools to screen children for neurodevelopment delay[313]. In 2013 the Centre for Paediatric Epidemiology and Biostatistics at UCL Institute of Child Health published their report on standardised tools that could measure a child’s overall neurodevelopment progress at age 2–2 1/2 years. This report was requested by the United Kingdom’s Department of Health (DH) to evaluate which assessment tool should be incorporated into the Healthy Child Programme (HCP) review, which is undertaken when children are between two and two and a half[318]. The authors concluded that both the ASQ and PEDS would meet the requirements, as set out by the DH, to measure children’s overall neurodevelopment skill at two years of age[318]. In reviewing the questionnaires, the ASQ-3 appears to be the most appropriate tool for performing a general multi-domain neurodevelopment screen for children aged two years. The ASQ-3 is acceptable to parents, and reports the child’s current skills, without using negative language or judgement on parental concerns. The ASQ-3 has also shown good sensitivity (75%) and specificity (81%) when compared to assessments directly administered by a trained professional[328]. In using the ASQ-3 it should be remembered that its ability to correctly identify which children do and do not need further assessment is less accurate for term, low risk children compared to high risk children.

In evaluating a child’s neurodevelopment progress, it is important to note any exposures or health conditions that could impact on a child’s neurodevelopment. The next sub-section will explore the effect of early nutrition in the first few months of life on neurodevelopment at 24 months of age.

2.5.2 Effect of early nutrition in the first few months of life on neurodevelopment at 24 months of age

Neurodevelopment starts within the first few weeks after conception and most neurodevelopmental processes are completed by three years of age. By a child’s second birthday, synapse formation and myelination is complete[329]. Early nutrition during this period is important for neurodevelopment; the rapid growth experienced by the brain makes it vulnerable to nutritional deficiencies[330]. The effect of malnutrition is influenced by the timing, severity and duration of exposure and the type of nutrition involved. Protein has a role in the neurologic processes (anatomy, chemistry and physiology and metabolism)[331] and has been shown to be an especially important nutritional exposure in the first 1,000 days[331]. Systematic reviews, that have met the PRISMA criteria and published between 2012-2017, reported that the growth rate, primarily the head circumference, experienced by infants also has an effect on their neurodevelopmental outcomes[332-334]. As highlighted by The Lancet’s 2017 series on ‘Advances in nutrition of the newborn infant’[335] early rapid growth, for preterm infants, is promoted to improve cognitive outcomes but currently this is at the expense of neonates developing components of the metabolic syndrome[335].

Infants born at high-risk of neurodevelopment delay due to prematurity have been shown to need additional protein beyond what breastmilk contains but so far studies examining the protein requirements have found inconsistent results on what the lower and higher levels of recommended protein intake should be to support healthy neurodevelopment[84, 90, 335-337]. Nearly all reviews and papers examining the effects on an infant’s milk diet or growth on their neurodevelopment outcomes have included infants who require interventional support to maintain and/or promote their health and development[338]. The populations most often utilized for these studies have the most to gain from any intervention. For term, healthy, singleton infants the effect of different milk diets on their development may not be as apparent as the population is at lower risk of neurodevelopmental delay.

The systematic reviews which support the WHO recommendation for exclusive breastfeeding in the first six months of life and continued breastfeeding up to 2 years and beyond included term, healthy children[1, 98-100, 208]. The EU Childhood Obesity Project[339] randomised infants, in the first year of life, to infant formula with either the legally lowest and highest permitted levels of protein. They[339] reported no differences in the neuropsychological test results of children at eight years of age based on their protein intake from their infant formula in the first year of life. Protein content, in the same study population, did affect BMI status at six years of age. Children assigned to the lower protein content infant formula had lower BMIs and a reduced risk of obesity compared to those children randomised to receive the higher protein content infant formula[60].

The EU Childhood Obesity Project was not the first study to examine both growth and neurodevelopment based on an infant’s milk diet. In 1929 Hoefer and Hardy published their findings which showed that breastfed (≤ 3months, 4-9 months and 10-20 months duration) infants in America were taller (weight is not provided) and younger when they started to walk and talk compared to infants who were formula-fed[340]. Studies, generally examined overall neurodevelopment or areas of neurodevelopment (i.e. cognitive, verbal and non-verbal language, fine and gross motor skills) but not all aspects of neurodevelopment.

Overall, studies reported a positive gradient relationship between increased duration of any breastfeeding and cognitive[341-348] and language[342-345] development. Studies differed in their findings on the effects of breastfeeding on motor (gross and fine) skills[343, 344, 349].

Overall, reviews looking at infant diet and neurodevelopment have reported that breastfeeding is the optimal nutrition to promote brain development[50, 331, 350, 351]. However, it is remarked that one of the biggest difficulties in teasing out the effects of early nutrition on neurodevelopment is the heterogeneity between studies. Another concern is the ability of studies to control for confounders, such as maternal intelligence[352-357].

The evidence, although mainly from studies including neonates at high risk of neurodevelopmental delay (i.e. preterm SGA infants), suggest that the predictors of growth could also be important for neurodevelopment and behaviour outcomes[21, 90]. In investigating the effect of a nutritional intervention on infant growth and what intervention should be applied, we also need to consider if this intervention will have a negative, neutral or positive effect on neurodevelopment.

2.6 Conclusion

The WHO recommendations on exclusive breastfeeding have been adopted by many international countries, including Ireland, Australia, Italy, Norway, UK and USA. Each of the national infant feeding studies used different methodology and this can make it difficult to evaluate whether countries are meeting their commitment to the WHO recommendations. The most common variation is in how breastfeeding is defined. Feeding surveys have not examined compliance with international guidelines on the recommended type of infant formula. All national infant feeding surveys examined if breastfeeding mothers were following WHO recommendations. This suggests that formula-fed infants are not receiving the same attention from healthcare researchers as their breastfeeding counter-parts. This is cause for concern, as the diet of all infants is important and by excluding the feeding patterns of formula-fed infants it remains unknown if they, and not just breastfed infants, are been fed according to international best-practice recommendations. It also means that the repercussions on infant health and development of inappropriate infant formula have not been addressed. This argument can also be applied to the reported health risks of not breastfeeding. All the meta-analysis[1, 98-100] exploring the recommended duration of exclusive breastfeeding did not explore if the type of infant formula (whey-based or not, protein levels etc) affects any of their findings.

The creation of the new WHO growth reference data has changed the language of child growth from describing how they grow to how children should grow. This now allows healthcare professionals to assess how well each child is growing compared to children, who are free from disease or ill-health, raised in optimal conditions. As it is an international growth chart, it also allows for direct comparisons between and within different countries and ethnic groups. NCSS data, currently, has not been assessed for its applicability and use in assessing neonatal size at birth[358]. It is unlikely that NCSS data will be used instead of the birth data provided by the MGRS (as NCSS is an extension of the MGRS) but will more likely be applied in the assessments of fetal growth.

Data on the antenatal predictors of infant growth in the first two years of life for term, singleton infants in a low-risk population are lacking. Studies which examined antenatal predictors such as maternal education[359, 360], ethnicity[95, 361, 362] and SES[363, 364] included pre-term infants or mothers with pre-existing conditions (i.e. diabetes mellitus) that could independently affect infant growth. Equally, there was limited data available on the effects of infant feeding in the first two months on growth in the first two years of life. Additional attention is needed to examine what are the antenatal and early infant feeding predictors of child growth in the first two years of life.

A number of studies investigating the Growth Acceleration Hypothesis have confirmed that rapid weight or growth in infancy, increases the risk of later on-set overweight and obesity for at least a third of the population. Rapid weight or growth in the first few months of life appeared to be the most sensitive risk factor for later onset overweight or obesity[182, 225, 229, 231, 233, 365, 366] and this is a time when infant growth is nearly exclusively dependent on infant diet[48]. The Early Protein Hypothesis, which is an extension of the Growth Acceleration Hypothesis was supported by the results from six out of 10 published studies, including all RCTs[54, 56, 57, 62, 243, 244, 247]. These studies have also reported that lower protein does not have a negative impact on neurodevelopment outcomes[335, 339]. This suggests that infant feeding is a potentially strong interventional strategy to prevent early excess weight gain.

In examining the role of an infant's milk diet on their growth, a 2014 meta-analysis highlighted that the method of defining breastfeeding (i.e. exclusive, mixed, any) in studies altered the estimation of the protective effect of breastfeeding, compared to infant formula, on obesity[10]. Most studies compare mixed feeding to formula feeding, and so the true effect of breastfeeding exclusively is unclear. Studies have also shown that how an infant responds to nutritional exposure is, in part, influenced by their sex[212, 367, 368]. Studies exploring the influence of diet and growth on later on-set overweight and obesity have not always clearly defined the milk diet of the participating infants or stratified their analysis by sex. The reliability and validity of the various methods to measure body composition are primarily based on Caucasian/ European descent populations from high income countries. The suitability of these methods for other ethnic groups has not been addressed[369].

There is little reference data available on the body composition of infants at birth, during the neonatal period, infancy and childhood [273, 274, 370] . All four studies measured body composition following delivery using the PEA POD® Infant Body Composition System with the Fomon Density Model. Two of the studies only included breastfeeding infants[273, 274] and one study excluded infants if failure to thrive was diagnosed and determined their required sample size based on calculations for single mean estimates[273]. Results from these studies have shown that there is strong sex and gestational age-related differences, in addition to geographical and ethic differences. This raises the question if a population-specific body composition reference data is required over one general body composition reference dataset[278]. Further attention is needed in exploring longitudinal body composition measurements in a clearly defined population, from multicentre sites, to address some of the current gaps in the literature.

Currently it is unclear if body composition differences exist between infants, based on their method of feeding, prior to them ever being fed. It also remains unknown what is the body composition of infants based on their defined and documented method of feeding, with other predictors of growth controlled for, at birth. Overall, there is a lack of studies examining the independent effect of infant feeding (clearly and consistently defined) on body composition at birth and later.

Neurodevelopmental progress is also an important consideration when examining growth in the first two years of life. Parent-completed questionnaires have shown reliability and validity against health-care administered questionnaires. One disadvantage to note would be the parent(s) literacy skills. Parent(s) with limited literacy skills may need the parental questionnaire to be administered via a health-care professional[371]. As studies investigated the validity of both the ASQ and PEDS questionnaires have shown the ASQ to have stronger sensitivity and specificity, compared to the PEDS, for children aged two years, it is the chosen instrument in this thesis to examine neurodevelopmental outcome.

Overall, some findings suggested that neurodevelopmental skills at two years of age are formed from a combination of environmental and lifestyle factors and not are the result of one particular influence. As systemic reviews for infants at risk of neurodevelopment delay have shown an association between growth and neurodevelopmental progress it is important, for this thesis, to include neurodevelopmental outcomes as part of the investigation in the effects of an infant’s milk diet on body composition and growth.

In conclusion, although many studies have examined the effect of breastfeeding on growth and development in the first two years of life, few have examined the effect of the composition of milk diet in non-breastfeeding infants. Little is known about body composition in infancy, and the effect of milk diet on body compositional change over the first few months of life. Therefore, the aim of this thesis is to address the current gaps in the literature examining the predictors and effect of an infant’s milk diet at two months on their body composition in the first two months of life, growth in the first two years of life and neurodevelopment at two years of life. This thesis will also examine if the body composition of infants are different at birth, prior to them receiving their very first feed.

Chapter 3: Methodology

3.1 Introduction to methods chapter

This chapter will give an overview of the methodology employed by the Cork BASELINE (Babies After SCOPE: Evaluating the Longitudinal Impact of Neurological and Nutritional Endpoints) Birth Cohort Study (www.baselinestudy.net) and the Screening for Pregnancy Endpoints (SCOPE) International Cohort Study (www.scopestudy.net) that was utilised in this thesis.

This chapter will also include general statistical methods undertaken throughout this thesis. The specific methodology details for each objective of this thesis are within the results section of this thesis. This chapter will end with a comparison of the study population to the Irish population.

3.2 Study procedures, relevant to this thesis, for both the SCOPE Ireland Study and the Cork BASELINE Birth Cohort Study

3.2.1 SCOPE Ireland Study

Between February 2007 and February 2011 healthy nulliparous women with singleton pregnancies, who were attending Cork University Maternity Hospital (CUMH), Cork, Ireland, were invited to participate in the SCOPE Ireland Study. Nulliparous who were not eligible to participate included those at high risk for pre-eclampsia, delivery of a small-for-gestational age neonate, spontaneous preterm birth due to underlying medical conditions (such as diabetes). Other exclusions that were applied included needing interventions (i.e.) aspirin that might modify the outcome of the pregnancy or experiencing three, or more, previous miscarriages or terminations of pregnancy.

At the first appointment (15±1 GW) eligibility and consent were confirmed. Questions asked included: Demographic, maternal obstetric history (including mother's own birthweight and gestational age at delivery), maternal medical history, information on current pregnancy (including hospital admissions), diet during pregnancy and lifestyle questions (including recreational drug use). At the 20GW assessment, mothers were again asked for information, since the last appointment, on any pregnancy events (including hospital admissions) and diet in pregnancy and lifestyle questions (including recreational drug use).

The maternal clinical examination at 15±1GW included blood pressure (with MICROLIFE BP 3AC1-2 Monitor), weight (standing without shoes or jacket, to the nearest one decimal point, using a hospital digital scale), height (standing without shoes to the nearest centimetre(cm), using a hospital wall chart) and hip (widest area of the buttocks in cm, using a disposable tape measure) circumference. At the 20GW assessment BP and weight were repeated.

Late pregnancy and intrapartum data were collected from maternal obstetric notes following delivery. Information collected included gestational diabetes mellitus (GDM) screening, results and treatment (if applicable) any medical concerns and last known weight. Neonatal course, including admission and events in the neonatal intensive care unit (NICU), birthweight, gestational age at delivery and method of feeding at discharge from the maternity hospital were also collected from the neonatal and obstetric notes.

3.2.2 SCOPE Ireland Study/Cork BASELINE Birth Cohort Study

All neonates (independent of gestational age at delivery, birth weight or any abnormalities) born to mothers who had participate in the SCOPE Ireland Study were eligible for inclusion in the Cork BASELINE Birth Cohort Study.

At the final SCOPE Study Ireland assessment which took place after delivery but prior to discharge, maternal consent for the infants to participate with the Cork BASELINE Birth Cohort Study was confirmed. If consent was obtained, this visit also served as the first assessment with the Cork BASELINE Birth Cohort Study. At this assessment each neonate had their naked weight (using the Seca 384 baby and floor scale which was then replaced by the electric scales on the PEA POD® Infant Body Composition System), length (using a neonatometer in cms), head (maximum occipito-frontal in cm), mid-arm (mid-way point between the edge of the acromion process and tip of the olecranon process in cm) and abdominal (just above the umbilicus) circumferences measured. From the 24th March 2009, the PEA POD® from Life Measurement Inc, Concord, CA using the Fomon Density Model also formed part of the assessment to capture neonatal body composition.

3.2.3 Cork BASELINE Birth Cohort Study

The first follow-up assessment occurred at two months. The same infant anthropometric and body composition measurements that were undertaken following delivery were also undertaken at the two month assessment; with the exception that abdominal circumference was not measured. A parental questionnaire was also completed. Questions asked included: demographic, environment during pregnancy (including maternal smoking history and household environment), environment during early life (including infant exposure to passive smoking), baby's nutrition, baby's health (including vaccination history, medicines given and why and admission to hospital).

Infants returned for anthropometric measurements and parental questionnaires at six, 12 and 24 months. At six and 12 months the same procedure was undertaken to obtain anthropometric measurements. Naked weight (kg) was taken using a Seca 384 baby and floor scale and length (cm) was measured using a Seca 210 Baby Length Measuring Mat. Head and mid-arm circumferences were measured as previously described. At two years of age additional measurements were taken. These included standing height (no shoes), instead of length and was measured using a Seca 206 Mechanical Measuring Tape. Additional measurements taken included: BP in mmhg and knee-ankle length, waist and hip circumferences were all measured in cms. Blood pressure was assessed using the appropriate child sized cuff that was at least 80 percent of the upper arm circumference on the child's left arm using a CARESCAPE V100 Vital Signs Monitor. Waist circumference was determined by placing the tape measure at the infant's navel and bringing it around the waist to the front. Hip circumference was assessed while the infant was undressed (for their weight measurement) and the tape measure was placed around the largest part of their buttocks.

The same parental questionnaires were asked at both the six and 12 months appointments. Information was collected on: baby's nutrition, baby's health (including admission to hospital, any illness and infections, any atopic symptoms) and any medications, list of foods introduced to baby's diet and when introduced and household environment (including current employment status and childcare arrangements). The parental questionnaires at 24 months remained largely unchanged from what was asked at the six and 12 months follow-up assessments. Questions on the infant's age when introduced to solid food/ having three meals a day and the number of meals and drinks the child had per day were removed.

Neurodevelopment screening at 24 months was undertaken using the parental-administered Ages & Stages Questionnaire, third edition, (ASQ-3)11. Parents were posted the ASQ-3 to complete prior to the child's 24 month assessment and to return the completed questionnaire when they presented with their child for the 24 month follow-up visit. For parents that did not bring the completed ASQ-3 with them to the 24 month follow-up visit they were provided with a stamped addressed envelope to post the questionnaire back to the Cork BASELINE Birth Cohort Study.

3.3 Ethics

The SCOPE Ireland Study is registered with Australian New Zealand Clinical Trials Registry (ACTRN12607000551493) and the Research Ethics Committee of the Cork Teaching Hospitals provided ethical approval for the SCOPE Ireland Study (ref ECM5(10) 05/02/08).

The Cork BASELINE Birth Cohort Study is registered with the United States National Institutes of Health Clinical Trials Registry (NCT01498965). Ethical approval, for the Cork BASELINE Birth Cohort, was also provided by the Research Ethics Committee of the Cork Teaching Hospitals (ref ECM5(9) 01/07/2008).

All study procedures were performed as per the guidelines from the Declaration of Helsinki. Prior to any assessment parent(s) were reminded of what was involved in the follow-up appointment and that all aspects of the assessment were completely voluntary. Informed consent was verbally re-checked at the start of each assessment and patient confidentiality was maintained by entering anonymised data into the on-line database. For the 24 month assessment, for as much as was possible, verbal child assent was also obtained. Assessments were stopped if requested by the parent(s), if the child became upset or did not comply to any of study producers.

3.4 Statistical methods

All statistical analysis was done using IBM SPSS Statistics Statistical (IBM Corp., Armonk, NY). Categorical variables were reported as absolute numbers and as percentages. Associations between categorical variables were examined using Pearson's chi-square test. Continuous variables were explored for their distribution. Parametric data are described by their mean and standard deviation (SD) and differences explored using Student's t-test. Non-parametric data are shown as median with their interquartile range (IQR) and examined using Mann Whitney U Test, unless otherwise stated.

Multivariable analysis with a continuous or categorical dependent variable were undertaken using linear and logistic regression, respectively. All estimates from multivariable analysis are reported alongside 95% confidence intervals. Statistical significance for all analysis was achieved with a p-value of ≤ 0.05 unless otherwise stated.

Directed acyclic graphs (DAGs) were used, with univariate analysis, to determine what co-variates needed to be added to each of the body composition, size and growth models and to minimise the number of confounders examined[372-374].

As results from the Cork BASELINE Birth Cohort Study[272] have shown that male and female infants differ in the body composition at birth and other studies[212, 367, 368] have suggested that female and male infants can respond differently to nutritional exposures all body composition, size and growth analysis was stratified by infant sex.

3.5 Study Sample and Irish Population

Outside of Dublin, CUMH has the highest number of births with over eight thousand births per year[370, 375]. Figures from the Central Statistics Office would suggest that, per year, nulliparous account for about three thousand deliveries in CUMH[375]. It should be noted that this figure cannot be broken down based on the inclusion/ exclusion criteria of the SCOPE Ireland Study. Overall, the SCOPE Ireland Study invited 2,579 nulliparous to participate and 1,774 (69%) were recruited. Out of the 1,774 mothers that formed part of the SCOPE Ireland Study 1,583 went on to deliver a live neonate in CUMH. All 1,583 mothers were approached to have their child participate in the Cork BASELINE Birth Cohort Study and 1,537/1583 (97.1%) signed the consent form.

Table 5 highlights the differences between the general maternal and neonatal population, as taken from Perinatal Statistics Report for 2010[376], to those who participated in the SCOPE Ireland Study and Cork BASELINE Birth Cohort Study. The 2010 Perinatal Statistics Report was selected because it was close to the half way point of recruitment for the SCOPE Ireland Study (which by extension was also the initial recruitment period for the Cork BASLINE Birth Cohort).

Table 5: General Population versus Study Population

	General Maternal & Neonatal Population	SCOPE Ireland Study & Cork BASELINE Birth Cohort Population
Maternal Population		
Average age (years) at delivery	31.5[a]	30.6
Percentage of mothers who were married	65%[b]	67%
Mother's nationality was Irish	75%[b]	77%
Percentage of mothers who were employers & managers	7%[b]	11%
Neonatal Population		
Average gestational age at delivery (weeks)	39[b]	39
Average birthweight (kg)	3.5[b]	3.4
Average number of days in hospital following delivery	4	4
Percentage of mothers reporting any breastfeeding at discharge	54.1%[c]	72.0%

a = includes still and live births; neonatal deaths by primiparous and multiparous, also includes singleton and multiple pregnancies

b = for all live singleton births; includes primiparous and multiparous

c = includes all neonates born to primiparous and multiparous

Chapter 4: Difference between body composition of formula and breastfed infants at birth

Abstract: Breastfeeding may reduce obesity risk, but this association could be confounded by characteristics of breastfeeding families. We thus investigated if body composition differs at birth among infants who were either exclusively breastfed or exclusively formula-fed. We hypothesized the two groups would differ in body composition, even at birth, prior to their actual post-natal feeding experience. Healthy, primiparous, singleton pregnancies were recruited at 15 weeks' gestation. Neonatal body composition was measured with the PEA POD® within 72 hours of delivery. Prospective infant feeding data was taken from maternity records and the two-month follow-up. Out of the 1152 infants recruited, 117 (10.2%) and 239 (20.7%) went on to be either exclusively breast- or formula-fed, respectively. Exclusively breastfed infants were heavier at birth, but their percentage fat mass was lower than that of exclusively formula-fed infants (covariate adjusted β = -1.91 percentage points of fat mass; 95% CI -2.82 to -1.01).

Introduction:

The degree to which breastfeeding truly affects later risk of obesity is unclear. It is difficult to determine if the effect is due to the maternal and social characteristics of families that breastfed[236, 377-380]. There is some evidence to support a causal link. Breastfed infants experience a slower early growth rate compared to formula-fed infants, which may reduce their risk of later onset obesity. Leptin, which directly affects satiety, is present in breastmilk but not infant formula[381]. The influence of the pre-natal environment, based on the method of feeding in the postnatal period, remain largely unexplored[382].

Evidence of pre-natal confounders should, at least in theory, be present at birth. Mothers that breastfed generally have a lower body mass index (BMI) during pregnancy[383], lower rates of smoking[384] and diabetes[385]. All these factors, independently, have been shown to influence both birthweight and body composition at birth[172]. As infants, who once born, become 'breastfed' or ‘formula-fed’, are exposed to different pre-natal conditions, we have hypothesised that differences may exist, between breastfed and formula-fed infants, at birth, prior to being fed[386-388]. We thus aimed to compare the body composition of term infants at birth who were subsequently exclusively breast- or formula-fed in the first two months of life.

Methods:

The study sample consisted of term (37+0 weeks’ gestation) infants only.

Maternal body mass index (BMI) was determined by dividing the recorded weight (kg) by height in m2. Body size classification was based on the World Health Organization BMI-based definitions for underweight (<18.5 kg/ m2), normal weight (≥18.5 to <25kg/ m2), overweight (≥25 to <30 kg/ m2) and obesity (≥ 30 kg/ m2)[389]. As per the recruiting hospital’s guidelines obese mothers were classified as being high risk for developing gestational diabetes mellitus (GDM)[390] and were referred for GDM screening at around 28 weeks’ gestation. Gestational weight gain from 15 weeks’ gestation up to delivery (GWG) was determined by subtracting the weight measured prior to labour from the weight recorded at 15 weeks’ gestation. The Institute of Medicine’s (IOM) criteria was used to determine if mothers exceeded their recommended weight gain[194].

Only infants that were exclusively breastfed or formula-fed from birth to two-months were included in the analysis. Our initial analysis of the data showed a gradient difference in anthropometric measurements and demographics between exclusively breastfed infants, infants that received both breastmilk and infant formula and exclusively formula-fed infants. Therefore, in this study we have excluded any infant that received both breastmilk and infant formula, either simultaneously or separately, at any stage in the first two months of life, to reduce cross-over effect on the results.

Results:

Consent was obtained for 1583 infants to participate in the Cork BASELINE Birth Cohort Study, and 1132 had their body composition measured prior to discharge from the maternity hospital following delivery. The number of drop-outs at delivery was 10(0.88%) and 71(6.27%) at two months, totalling 81(7.15%) infants. Out of the remaining 1051 participants, 24 were preterm (≤36+6 weeks' gestation), 77 were missing method of feeding at two months and 594 term infants had a feeding history did not meet this study's inclusion criteria due to mixed feeding. This left a final sample of 356 infants: 117 (32.9%) exclusively breastfed and 239 (67.1%) exclusively formula-fed. We did not find any differences in maternal BMI, reported rates of smoking in pregnancy, GDM, maternal employment or infant sex and weight-for-length z-score at birth between infants that did and did not have their body composition assessed following delivery. All but one infant (in the formula-feeding group) had their growth scan and all infants had their body composition assessed.

Mothers who breastfed differed significantly from those who formula fed in several clinical variables (Table 6). Reported rates of smoking during pregnancy differed between mothers who breast- and formula-fed their infants (1.7% and 13.8% respectively; X2(1) = 12.97, N = 356, $p < 0.001$). Breastfeeding mothers had lower mean(SD) BMI at 15 weeks' gestation compared to mothers who formula-fed their infants (23.29(3.12) versus 25.53(4.28); t(351) = -4.97, $p < 0.001$). Nearly five times as many mothers who went on to exclusively formula-feed their infants required screening for GDM, based on their BMI at 15 weeks' gestation, compared to breastfeeding mothers (17.2% and 3.5%, respectively; X2(1) = 13.18, N=353, $p<0.001$). Formula feeding mothers also had significantly higher systolic blood pressure (105.15(10.08) vs. 103.11(9.78) mmHg respectively; t(354) = -2.70, p = 0.007), and larger waist (82.30(9.80) vs. 77.22(6.80) cm; t(354) = -5.04, $p < 0.001$) and hip circumferences (98.33(8.91) vs. (94.22(7.61) cm, t(353) = -4.28, $p < 0.001$) at 15 weeks' gestation. These differences persisted up to delivery.

Socio-economic differences were also observed. Nearly twice as many breastfeeding mothers had a tertiary education compared to mothers who formula-fed their infants. Although rates of employment did not differ between the groups, 76.1% of breastfeeding mothers were classified as managers or professionals compared to 45.2% of formula-feeding mothers (X2(1) = 30.31, N = 356, p=<0.001).

In the antenatal growth scan (Table 6), breastfed infants had a significantly smaller abdominal circumference compared to formula-fed infants (157.3(9.67) vs. 160.3(10.19) mm; t(355) = -2.66, p = 0.008).

At birth, for both sexes, infants who progressed to exclusive breastfeeding were heavier when compared to formula-fed infants. Female infants, in particular were heavier than their formula fed counterparts (3.51(0.36) vs 3.36(0.42) kg; t(169) = 2.37, p = 0.02), whilst in males the difference was not significant (3.62(0.49) vs 3.54(0.44) kg, t(183) = 0.98, p = 0.33). Although breastfed infants were heavier at birth, they had significantly lower fat mass (FM) (0.34(0.14) vs. 0.41(0.17) kg; t(356) = -4.13, p < 0.001), percentage fat mass (%FM) (10.01(3.71) versus 12.05(4.06)%; t(356) = -4.59, p < 0.001), and lower percentage fat free mass (%FMM) (87.95(4.06) vs. 89.99(3.71)%; t(356) = 4.59, p < 0.001) compared to formula-fed infants. These differences remained when stratified by infant sex (Table 6). Head, arm and abdomen circumferences; length; and fat free mass (FMM) were similar for male and female infants across both feeding groups.

In multiple linear regression, being born to a mother that will breastfeed, compared to a mother that will formula feed, significantly reduced the %FM, for both male and female infants, when controlled for gestational weeks at delivery, maternal profession and BMI at 15 weeks' gestation, Table 7.

Discussion:

We have shown that body composition differences are present at birth between infants who progressed to exclusive breastfeeding compared to infants that never receive breastmilk and are exclusively formula-fed from birth. Results indicated that infants, based on their mothers' intended method of infant feeding, do differ at birth. We have also shown that there are significant anthropometric and socioeconomic differences between breastfeeding and formula-feeding mothers. This is an important finding as it shows that differences exist prior to the exposure, highlighting that the effects of breastfeeding on obesity may be confounded by maternal lifestyle and pre-natal environmental factors.

The factors driving this variability are unclear, but are likely to be a confluence of maternal diet, activity and stress levels, affecting placental nutritional delivery and placental growth hormones. Why these differ in mothers who successfully breastfeed to two months compared to those who bottle feed from birth is also unclear, but is likely to be a combination of health education, and nutritional awareness. Our small study was not able to examine each of these factors in detail, but raises important questions about when obesity intervention needs to begin. Should we define the birth weight/body composition of breastfed infants as the ideal? This would mean that body composition reference ranges would shift to almost 1.5% lower than currently published when all feeding types are included[272].

Previous studies have shown that maternal smoking significantly reduces infant birthweight and FFM but not FM[176, 177]. We also found that maternal smoking significantly reduced infant body mass and FFM but did not affect FM. However, we were unable to explore the effect of maternal smoking, based on how the infants were fed, on infant body composition, as only two exclusively breastfeeding mothers reported smoking in pregnancy.

GDM screening in the SCOPE Ireland Study was based on the presence of one or more risk factors, including high BMI. Mothers whose clinicians placed them at high risk for GDM due to their BMI gave birth to infants with increased %FM, compared to mothers with a BMI that did not warrant GDM screening. Notably, one previous study has shown that the relationship between maternal BMI and neonatal body composition is negated if adjusted for the maternal glucose tolerance test and fasting glucose levels from 36-38 gestational weeks'[288]. We were not able to explore the relative importance of these exposures as fasting blood glucose levels were not available in the cohort, but only those screened for GDM.

Limitations to this study include that it is a secondary data analysis. The Cork BASELINE Birth Cohort Study was established to examine the body composition of infants but not based on method of feeding at two months of age. We did not collect data on maternal health beliefs and breastfeeding intentions. As with all observational studies, caution should be applied in interpreting the results due to risks of confounding and selection bias. Our study sample came from a large, prospective, population-based cohort which used reference methods to capture neonatal body composition. All eligible infants were included in this analysis. Our sample size was reduced as the Cork BASELINE Birth Cohort Study was established prior to the installation of the PEA POD® Infant Body Composition System. Due to this delay over a fifth (27%) of the cohort did not have their body composition assessed[370] and were therefore ineligible for this paper.

Our paper did find that the birth weight and body composition of infants who are breastfed successfully by their mothers to 2 months differs significantly from that of infants exclusively formula fed from birth. Our analysis raises important questions about the true post-natal effect of breastfeeding as the intra-uterine environment of these infants may have a significant role to play. The differing obesity risk and early growth trajectory seen in breastfed infants, may in part be due to differences in their intra-uterine exposures, irrespective of their early postnatal diet.

Table 6: Study Sample Characteristics

	All Formula-Fed	All Breastfed	P-Value	Male Formula-Fed	Male Breastfed	P-Value	Female Formula-Fed	Female Breastfed	P-Value
Sample Size	*239 (100%)*	*117 (100%)*	*N/A*	*131/239 (54.8%)*	*54/117 (46.2%)*	*N/A*	*108/239 (45.2%)*	*63/117 (53.8%)*	*N/A*
Maternal BMI at 15 weeks' gestation	25.53 (4.28)	23.29 (3.12)	<0.001	25.48 (4.61)	23.22 (2.68)	0.001	25.58 (3.86)	23.36 (3.48)	<0.001
Maternal weight gain exceeded IOM recommendations: Yes	69 (42.6%)	34 (35.8%)	0.34	36 (40.4%)	14 (33.3%)	0.38	33 (45.2%)	20 (37.7%)	0.54
Maternal Tertiary Education: Yes	82 (34.3%)	80 (68.4%)	<0.001	35 (26.7%)	34 (63.0%)	<0.001	47 (43.5%)	46 (73.0%)	<0.001

	All Formula-Fed	All Breastfed	P-Value	Male Formula-Fed	Male Breastfed	P-Value	Female Formula-Fed	Female Breastfed	P-Value
Maternal employment: Yes	218 (91.8%)	102 (87.2%)	0.16	122 (93.1%)	47 (87.0%)	0.18	96 (88.9%)	55 (87.3%)	0.55
Biparietal diameter (mm) from fetal growth scan	49.86 (2.92)	48.95 (3.21)	0.04	50.40 (2.77)	50.24 (3.22)	0.73	48.79 (2.89)	47.80 (2.74)	0.04
Head circumference (mm) from fetal growth scan	185.63 (9.64)	182.51 (10.63)	0.007	187.52 (9.01)	186.06 (10.71)	0.35	183.33 (9.91)	179.38 (9.59)	0.02
Abdominal circumference (mm) from fetal growth scan	160.32 (10.19)	157.30 (9.67)	0.008	161.86 (9.81)	159.31 (10.03)	0.13	158.44 (10.37)	155.55 (9.06)	0.11
Femur length (mm) from fetal growth scan	34.08 (2.36)	33.43 (2.50)	0.003	34.15 (2.38)	33.85 (2.54)	0.48	34.00 (2.35)	33.06 (2.43)	0.02

	All Formula-Fed	All Breastfed	P-Value	Male Formula-Fed	Male Breastfed	P-Value	Female Formula-Fed	Female Breastfed	P-Value
Gestational age (weeks) at delivery	39.65 (1.13)	39.97 (1.05)	0.01	39.71 (1.16)	40.11 (1.02)	0.03	39.58 (1.10)	39.86 (1.07)	0.10
Infant birthweight (kg)	3.46 (0.44)	3.56 (0.42)	0.04	3.54 (0.44)	3.62 (0.48)	0.33	3.36 (0.42)	3.51 (0.36)	0.02
Infant length (cm)	50.27 (2.01)	50.60 (1.89)	0.18	50.75 (1.97)	50.96 (2.05)	0.53	49.69 (1.90)	50.28 (1.69)	0.06
Infant fat mass (kg)	0.41 (0.17)	0.34 (0.14)	<0.001	0.41 (0.19)	0.30 (0.14)	<0.001	0.42 (0.16)	0.37 (0.14)	0.032
Infant fat mass (%)	12.05 (4.06)	10.01 (3.71)	<0.001	11.48 (4.33)	8.60 (3.46)	<0.001	12.74 (3.60)	11.21 (3.50)	0.006
Infant fat free mass (kg)	2.95 (0.33)	2.99 (0.35)	0.20	3.05 (0.32)	3.08 (0.39)	0.51	2.83 (0.32)	2.92 (0.29)	0.05

Table 7: Multiple Linear Regression For Neonatal %FM

	β (95% CI)	P-Value
ALL infants		
(Constant)	1.68 (-13.02, 16.38)	0.82
Female Infant (versus male infant)	1.77 (0.97, 2.58)	<0.001
Exclusively Breastfed (versus exclusive formula feeding)	-1.91 (-2.82, -1.09)	<0.001
Gestational weeks at delivery	0.18 (-0.18, 0.54)	0.33
Maternal occupation is manager/professional	-0.50 (-1.36, 0.36)	0.25
Maternal BMI at 15 weeks’ gestation	0.10 (0.002, 0.21)	0.05
MALE infants		
Constant	-5.11 (-26.77, 16.56)	0.64
Exclusively Breastfed (versus exclusive formula feeding)	-2.53 (-3.92, -1.14)	<0.001
Gestational weeks at delivery	0.34 (-0.20, 0.87)	0.21
Maternal occupation is manager/professional	-0.60 (-1.93, 0.73)	0.38
Maternal BMI at 15 weeks’ gestation	0.13 (-0.01, 0.27)	0.07
FEMALE infants		
Constant	10.07 (-10.14, 30.28)	0.33
Exclusively Breastfed (versus exclusive formula feeding)	-1.33 (-2.51, -0.15)	0.03
Gestational weeks at delivery	0.03 (-0.47, 0.53)	0.92
Maternal occupation is manager/professional	-0.46 (-1.59, 0.67)	0.42
Maternal BMI at 15 weeks’ gestation	0.07 (-0.07, 0.22)	0.33

Supplementary Tables_1:

Background:

Our primary analysis only focused on infants that were either exclusively breastfed or formula-fed throughout the first two months of life. We excluded any mixed feeders (those that received both breastmilk and infant formula during the first two months of life) from the original analysis because:

- We did not collect information on duration of exclusive breastfeeding. So, we do not know, and are therefore unable to control for, duration of exclusive breastfeeding for those that were introduced to infant formula either in addition to continuing to receive breastmilk or stopped receiving any breastmilk altogether.
- We are also unable to tell how many infants were originally exclusively breastfed, introduced to infant formula in addition to receiving breastmilk and by two months were then exclusively fed infant formula.
- The data we collected on breastfeeding duration was when the infant was last ever breastfed.
- For those that received both breastmilk and infant formula we do not know how many feeds of breastmilk and infant formula per day, during the two-month period, were given to the infants. Therefore, some mixed feeders may have only received one bottle of infant formula per day and others may have received only one breastfed a day

All these points should be taken into consideration when reading the supplementary tables.

Methods:

As with the primary analysis we categorized infants based on medical reports from the maternity hospital, and parental reports of infant feeding at two months of age. Infants that received both breastmilk and infant formula were categorized as ‘mixed feeders’. Infants were grouped as mixed-fed (both breastmilk and infant formula) from birth, mixed-fed by two months of age (independent of how they were fed in the maternity hospital) and formula-fed by two months of age (independent if were mixed feeders or exclusively breastfed in the maternity hospital).

Categorical variables were reported as absolute numbers and as percentages. Associations between categorical variables were examined using Pearson's chi-square test. Continuous variables were described by their mean and standard deviation (SD); and differences between the feeding groups were tested using one-way analysis of variance (ANOVA) with Tukey post hoc test. The exclusively breastfed group (as reported in the main text) is the reference group for all analysis. The multivariable analysis was undertaken using linear regression and all estimates are reported alongside 95% confidence intervals. For the multivariable analysis we included the same variables from the primary analysis presented in the main paper.

Table 8: Study Sample Characteristics for All Participants

	Exclusively Breastfed	Mixed-fed from birth	Mixed-fed by two months	Formula-fed by two months	P-Value (Breastfed versus Mixed-fed from birth)	P-Value (Breastfed versus Mixed-fed by two months)	P-Value (Breastfed versus Formula-fed by two months)
Sample Size	*117 (100%)*	*386 (100%)*	*207 (100%)*	*39 (100%)*	*N/A*	*N/A*	*N/A*
Maternal BMI at 15 weeks' gestation	23.29 (3.12)	25.11 (4.14)	24.38 (3.52)	26.68 (6.05)	<0.001	0.08	<0.001
Maternal weight gain exceeded IOM recommendations: Yes	34 (35.8%)	115 (42.8%)	57 (38.3%)	11 (37.9%)	0.27	0.76	0.88
Maternal Tertiary Education: Yes	80 (68.4%)	229 (59.3%)	129 (62.3%)	16 (41.0%)	0.07	0.25	0.002
Maternal employment: Yes	102 (87.2%)	357 (92.5%)	182 (87.9%)	33 (84.6%)	0.04	0.70	0.78

	Exclusively Breastfed	Mixed-fed from birth	Mixed-fed by two months	Formula-fed by two months	P-Value (Breastfed versus Mixed-fed from birth)	P-Value (Breastfed versus Mixed-fed by two months)	P-Value (Breastfed versus Formula-fed by two months)
Biparietal diameter (mm) from fetal growth scan	48.95 (3.21)	49.50 (2.87)	49.10 (2.85)	49.79 (3.29)	0.31	0.97	0.43
Head circumference (mm) from fetal growth scan	182.51 (10.63)	185.17 (9.70)	183.79 (9.68)	185.03 (11.65)	0.06	0.69	0.53
Abdominal circumference (mm) from fetal growth scan	157.30 (9.67)	159.77 (9.97)	158.98 (9.74)	161.05 (11.95)	0.11	0.53	0.20
Femur length (mm) from fetal growth scan	33.43 (2.50)	34.04 (2.63)	33.86 (2.44)	34.23 (2.53)	0.13	0.51	0.35
Gestational age (weeks) at delivery	39.65 (1.13)	39.75 (1.15)	39.83 (1.17)	39.69 (1.26)	0.25	0.70	0.54
Infant birthweight (kg)	3.56 (0.42)	3.55 (0.49)	3.52 (0.40)	3.60 (0.51)	1.00	0.83	0.98

	Exclusively Breastfed	Mixed-fed from birth	Mixed-fed by two months	Formula-fed by two months	P-Value (Breastfed versus Mixed-fed from birth)	P-Value (Breastfed versus Mixed-fed by two months)	P-Value (Breastfed versus Formula-fed by two months)
Infant length (cm)	50.60 (1.89)	50.57 (2.09)	50.37 (1.85)	50.84 (2.08)	1.00	0.77	0.91
Infant fat mass (kg)	0.34 (0.14)	0.38 (0.18)	0.36 (0.15)	0.43 (0.18)	0.10	0.54	0.02
Infant fat mass (%)	10.01 (3.71)	10.98 (4.16)	10.82 (3.95)	12.10 (3.91)	0.11	0.34	0.03
Infant fat free mass (kg)	2.99 (0.35)	2.97 (0.35)	2.95 (0.31)	2.96 (0.53)	0.88	0.61	0.93

Table 9: Study Sample Characteristics for Male Participants

	Breastfed	Mixed-fed from birth	Mixed-fed by two months	Formula-fed by two months	P-Value (Breastfed versus Mixed-fed from birth)	P-Value (Breastfed versus Mixed-fed by two months)	P-Value (Breastfed versus Formula-fed by two months)
Sample Size	*54 (100%)*	*199 (100%)*	*99 (100%)*	*20 (100%)*	*N/A*	*N/A*	*N/A*
Maternal BMI at 15 weeks' gestation	23.22 (2.68)	25.04 (3.99)	24.72 (3.52)	25.92 (6.45)	0.01	0.11	0.04
Maternal weight gain exceeded IOM recommendations: Yes	14 (33.3%)	59 (42.4%)	27 (39.7%)	6 (33.3%)	0.25	0.45	0.95
Maternal Tertiary Education: Yes	34 (63.0%)	125 (62.8%)	65 (65.7%)	8 (40.0%)	0.98	0.74	0.08
Maternal employment: Yes	47 (87.0%)	184 (92.5%)	87 (87.9%)	18 (90.0%)	0.21	0.88	0.73
Biparietal diameter (mm) from fetal growth scan	50.24 (3.22)	50.08 (2.82)	49.49 (2.90)	49.74 (3.40)	0.98	0.44	0.92

	Breastfed	Mixed-fed from birth	Mixed-fed by two months	Formula-fed by two months	P-Value (Breastfed versus Mixed-fed from birth)	P-Value (Breastfed versus Mixed-fed by two months)	P-Value (Breastfed versus Formula-fed by two months)
Head circumference (mm) from fetal growth scan	186.06 (10.71)	186.81 (9.56)	184.77 (9.96)	184.95 (9.96)	0.96	0.88	0.98
Abdominal circumference (mm) from fetal growth scan	159.31 (10.03)	160.87 (9.65)	159.44 (10.05)	161.10 (14.04)	0.75	1.00	0.91
Femur length (mm) from fetal growth scan	33.85 (2.54)	33.99 (2.62)	33.58 (2.46)	33.50 (2.14)	0.99	0.93	0.95
Gestational age (weeks) at delivery	40.11 (1.02)	39.69 (1.19)	39.72 (1.22)	39.90 (1.17)	0.10	0.20	0.90
Infant birthweight (kg)	3.62 (0.48)	3.53 (0.48)	3.55 (0.41)	3.57 (0.53)	0.99	0.81	0.97
Infant length (cm)	50.96 (2.05)	50.85 (2.01)	50.57 (2.01)	51.08 (2.17)	0.98	0.66	1.00
Infant fat mass (kg)	0.30 (0.14)	0.35 (0.17)	0.33 (0.15)	0.41 (0.17)	0.09	0.68	0.04

	Breastfed	Mixed-fed from birth	Mixed-fed by two months	Formula-fed by two months	P-Value (Breastfed versus Mixed-fed from birth)	P-Value (Breastfed versus Mixed-fed by two months)	P-Value (Breastfed versus Formula-fed by two months)
Infant fat mass (%)	8.60 (3.46)	10.15 (3.78)	9.59 (3.66)	11.59 (3.57)	0.04	0.42	0.01
Infant fat free mass (kg)	3.08 (0.39)	3.03 (0.35)	3.00 (0.30)	3.02 (0.39)	0.75	0.53	0.90

Table 10: Study Sample Characteristics for Female Participants

	Breastfed	Mixed-fed from birth	Mixed-fed by two months of age	Formula-fed by two months of age	P-Value (Breastfed versus Mixed-fed from birth)	P-Value (Breastfed versus Mixed-fed by two months)	P-Value (Breastfed versus Formula-fed by two months)
Sample Size	*63 (100%)*	*187 (100%)*	*108 (100%)*	*19 (100%)*	*N/A*	*N/A*	*N/A*
Maternal BMI at 15 weeks' gestation	23.36 (3.48)	25.19 (4.30)	24.07 (3.50)	27.47 (5.67)	0.01	0.68	0.001
Maternal weight gain exceeded IOM recommendations	20 (37.7%)	56 (43.1%)	30 (37.0%)	5 (45.5%)	0.63	0.79	0.70
Maternal Tertiary Education: Yes	46 (73.0%)	104 (55.6%)	64 (59.3%)	8 (42.1%)	0.01	0.06	0.01
Maternal employment: Yes	55 (87.3%)	173 (92.5%)	95 (88.0%)	15 (78.9%)	0.12	0.70	0.46

	Breastfed	Mixed-fed from birth	Mixed-fed by two months of age	Formula-fed by two months of age	P-Value (Breastfed versus Mixed-fed from birth)	P-Value (Breastfed versus Mixed-fed by two months)	P-Value (Breastfed versus Formula-fed by two months)
Biparietal diameter (mm) from fetal growth scan	47.80 (2.74)	48.88 (2.80)	48.75 (2.77)	49.84 (3.27)	0.06	0.18	0.03
Head circumference (mm) from fetal growth scan	179.38 (9.59)	183.41 (9.57)	182.90 (9.38)	185.11 (9.91)	0.03	0.11	0.11
Abdominal circumference (mm) from fetal growth scan	155.55 (9.06)	158.62 (10.20)	158.56 (9.49)	161.00 (9.67)	0.20	0.28	0.18
Femur length (mm) from fetal growth scan at	33.06 (2.43)	34.10 (2.64)	34.11 (2.40)	35.00 (2.73)	0.04	0.07	0.03
Gestational age (weeks) at delivery	39.58 (1.10)	39.81 (1.10)	39.94 (1.11)	39.47 (1.35)	0.99	0.97	0.55

	Breastfed	Mixed-fed from birth	Mixed-fed by two months of age	Formula-fed by two months of age	P-Value (Breastfed versus Mixed-fed from birth)	P-Value (Breastfed versus Mixed-fed by two months)	P-Value (Breastfed versus Formula-fed by two months)
Infant birthweight (kg)	3.51 (0.36)	3.50 (0.50)	3.49 (0.39)	3.63 (0.48)	1.00	0.99	0.78
Infant length (cm)	50.28 (1.69)	50.27 (2.13)	50.19 (1.69)	50.58 (2.00)	1.00	0.99	0.93
Infant fat mass (kg)	0.37 (0.14)	0.41 (0.19)	0.40 (0.15)	0.45 (0.19)	0.57	0.79	0.33
Infant fat mass (%)	11.21 (3.50)	11.88 (4.38)	11.95 (3.89)	12.64 (4.27)	0.69	0.68	0.55
Infant fat free mass (kg)	2.92 (0.29)	2.91 (0.34)	2.90 (0.30)	2.89 (0.65)	0.98	0.95	0.99

Table 11a: Multiple Linear Regression For Neonatal %FM

	β (95% CI)	P-Value
ALL infants		
(Constant)	-9.32 (-21.51, 2.86)	0.13
Female Infant (versus male infant)	1.90 (1.21, 2.58)	<0.001
Exclusively Breastfed (versus mixed-fed from birth)	-1.14 (-1.97, -0.31)	0.007
Gestational weeks at delivery	0.48 (0.18, 0.79)	0.002
Maternal occupation is manager/professional	-0.33 (-1.01, 0.36)	0.35
Maternal BMI at 15 weeks' gestation	0.02 (-0.73, 0.10)	0.74
MALE infants		
Constant	1.33 (-14.64, 17.30)	0.87
Exclusively Breastfed (versus mixed-fed from birth)	-1.60 (-2.75, -0.44)	0.007
Gestational weeks at delivery	0.22 (-0.18, 0.62)	0.28
Maternal occupation is manager/professional	-0.43 (-1.35, 0.49)	0.36
Maternal BMI at 15 weeks' gestation	0.01 (-0.11, 0.14)	0.83
FEMALE infants		
Constant	-20.76 (-39.49, -2.04)	0.03
Exclusively Breastfed (versus mixed-fed from birth)	-0.67 (-1.86, 0.52)	0.27
Gestational weeks at delivery	0.82 (0.34, 1.29)	0.001
Maternal occupation is manager/professional	-0.21 (-1.23, 0.82)	0.69
Maternal BMI at 15 weeks' gestation	0.01 (-0.12, 0.14)	0.87

Table 11b: Multiple Linear Regression For Neonatal %FM

	β (95% CI)	P-Value
ALL infants		
(Constant)	5.11 (-9.27, 19.48)	0.49
Female Infant (versus male infant)	2.43 (1.63, 3.24)	<0.001
Exclusively Breastfed (versus mixed-fed by two months old)	-0.87 (-1.71, -0.02)	0.04
Gestational weeks at delivery	0.10 (-0.26, 0.46)	0.59
Maternal occupation is manager/professional	-0.34 (-1.15, 0.47)	0.41
Maternal BMI at 15 weeks' gestation	-0.009 (-0.13, 0.11)	0.88
MALE infants		
Constant	4.96 (-15.06, 24.97)	0.63
Exclusively Breastfed (versus mixed-fed by two months old)	-1.10 (-2.34, 0.14)	0.08
Gestational weeks at delivery	0.16 (-0.34, 0.66)	0.53
Maternal occupation is manager/professional	-1.11 (-2.25, 0.04)	0.06
Maternal BMI at 15 weeks' gestation	-0.04 (-0.22, 0.13)	0.63
FEMALE infants		
Constant	9.62 (-11.29, 30.52)	0.37
Exclusively Breastfed (versus mixed-fed by two months old)	-0.68 (-1.87, 0.50)	0.26
Gestational weeks at delivery	0.04 (-0.49, 0.58)	0.87
Maternal occupation is manager/professional	0.34 (-0.81, 1.49)	0.56
Maternal BMI at 15 weeks' gestation	0.02 (-0.15, 0.18)	0.84

Table 11c: Multiple Linear Regression For Neonatal %FM

	β (95% CI)	P-Value
ALL infants		
(Constant)	15.78 (-5.02, 36.58)	0.14
Female Infant (versus male infant)	2.20 (1.05, 3.35)	<0.001
Exclusively Breastfed (versus formula-fed by two months old)	-2.18 (-3.60, -0.76)	0.003
Gestational weeks at delivery	-0.10 (-0.63, 0.44)	0.73
Maternal occupation is manager/professional	-0.67 (-1.82, 0.48)	0.25
Maternal BMI at 15 weeks' gestation	-0.03 (-0.17, 0.12)	0.70
MALE infants		
Constant	17.15 (-13.58, 47.89)	0.27
Exclusively Breastfed (versus formula-fed by two months old)	-2.65 (-4.57, -0.74)	0.007
Gestational weeks at delivery	-0.15 (-0.94, 0.64)	0.71
Maternal occupation is manager/professional	-1.26 (-2.89, 0.38)	0.13
Maternal BMI at 15 weeks' gestation	0.03 (-0.18, 0.24)	0.76
FEMALE infants		
Constant	17.66 (-11.12, 46.45)	0.23
Exclusively Breastfed (versus formula-fed by two months old)	-1.59 (-3.74, 0.57)	0.15
Gestational weeks at delivery	-0.09 (-0.83, 0.65)	0.81
Maternal occupation is manager/professional	-0.15 (-1.82, 1.51)	0.86
Maternal BMI at 15 weeks' gestation	-0.05 (-0.26, 0.16)	0.61

Supplementary Tables_2:

Background:

Our primary analysis involved using Directed Acyclic Graphs (DAGs), with univariate analysis, to help identify where adjusting for a possible confounder would create collider bias. DAGs also allow you to adjust for the minimum number of variables which helps against introducing confounder bias. To allow greater comparison to the results of other studies in this section all variables that were statistically significant between the feeding groups were included in the multivariate analysis.

Also, in the primary multivariate analysis data was first adjusted and then stratified by infant sex but no interaction effect model was undertaken. In this supplementary section interaction models using sex and infant feeding were also undertaken to examine how much they moderated the relationship between fetal size and percentage fat mass at birth.

Methods:

Feeding group remains the same as it did in the primary analysis: only infants that were exclusively breastfed or formula-fed from birth to two-months were included in the analysis.

Multivariable analysis was undertaken using linear regression and all estimates are reported alongside 95% confidence intervals. All variables that were statistically significant in univariate analysis between the feeding groups were included in the model.

The multivariate analysis was first adjusted and then stratified by infant sex. The final multivariate analysis included the interaction model. The effect term used, in the interaction model, was based on sex*infant feeding and included the following fetal assessments: biparietal diameter, head circumference and abdominal circumference.

Table 12a: Multiple Linear Regression For Neonatal %FM (all infants)

	β (95% CI)	P-Value
ALL infants		
(Constant)	-4.83 (-22.29, 12.64)	0.59
Female Infant (versus male infant)	1.59 (0.74, 2.44)	<0.001
Exclusively Breastfed (versus formula-fed by two months old)	1.81 (0.86, 2.77)	<0.001
Gestational weeks at delivery	0.31 (-0.05, 0.67)	0.09
Maternal occupation is manager/professional	-0.62 (-1.51, 0.26)	0.17
Maternal BMI at 15 weeks' gestation	0.03 (-0.15, 0.22)	0.73
Mother had completed tertiary education (versus not completed tertiary education)	0.13 (-0.79, 1.05)	0.78
Maternal waist (cm) at 15 gestational weeks'	0.01 (-0.08, 0.10)	0.82
Maternal hip (cm) at 15 gestational weeks'	0.01 (-0.03, 0.05)	0.48
Number of cigarettes smoked at 20 gestational weeks'	0.07 (-0.09, 0.23)	0.38
Fetal biparietal diameter at 20 gestational weeks'	-0.15 (-0.47, 0.17)	0.37
Fetal head circumference at 20 gestational weeks'	-0.04 (-0.14, 0.07)	0.53
Fetal abdominal circumference at 20 gestational weeks'	0.07 (-0.001, 0.13)	0.05
Fetal femur length at 20 gestational weeks'	0.07 (-0.19, 0.33)	0.6

Table 12b: Multiple Linear Regression For Neonatal %FM (male infants)

	β (95% CI)	P-Value
MALE infants		
(Constant)	-17.45 (-42.45, 75.55)	0.17
Exclusively Breastfed (versus formula-fed by two months old)	2.57 (1.16, 3.99)	<0.001
Gestational weeks at delivery	0.44 (-0.08, 0.95)	0.10
Maternal occupation is manager/professional	-1.16 (-2.42, 0.11)	0.07
Maternal BMI at 15 weeks' gestation	-0.008 (-0.31, 0.30)	0.96
Mother had completed tertiary education (versus not completed tertiary education)	1.09 (-0.26, 2.45)	0.11
Maternal waist (cm) at 15 gestational weeks'	-0.006 (-0.14, 0.13)	0.93
Maternal hip (cm) at 15 gestational weeks'	0.06 (-0.06, 0.19)	0.34
Number of cigarettes smoked at 20 gestational weeks'	0.17 (-0.05, 0.39)	0.12
Fetal biparietal diameter at 20 gestational weeks'	-0.02 (-0.46, 0.43)	0.94
Fetal head circumference at 20 gestational weeks'	-0.07 (-0.23, 0.09)	0.37
Fetal abdominal circumference at 20 gestational weeks'	0.06 (-0.03, 0.15)	0.18
Fetal femur length at 20 gestational weeks'	0.24 (-0.13, 0.60)	0.20

Table 12c: Multiple Linear Regression For Neonatal %FM (female infants)

	β (95% CI)	P-Value
FEMALE infants		
(Constant)	4.99 (-19.85, 29.83)	0.69
Exclusively Breastfed (versus formula-fed by two months old)	1.22 (-0.09, 2.53)	0.07
Gestational weeks at delivery	0.24 (-0.26, 0.75)	0.35
Maternal occupation is manager/professional	-0.18 (-1.46, 1.11)	0.79
Maternal BMI at 15 weeks' gestation	0.02 (-0.22, 0.26)	0.87
Mother had completed tertiary education (versus not completed tertiary education)	-0.69 (-1.98, 0.60)	0.29
Maternal waist (cm) at 15 gestational weeks'	0.001 (-0.11, 0.12)	0.98
Maternal hip (cm) at 15 gestational weeks'	0.005 (-0.04, 0.05)	0.83
Number of cigarettes smoked at 20 gestational weeks'	-0.004 (-0.24, 0.23)	0.98
Fetal biparietal diameter at 20 gestational weeks'	-0.25 (-0.73, 0.23)	0.30
Fetal head circumference at 20 gestational weeks'	-0.005 (-0.16, 0.15)	0.95
Fetal abdominal circumference at 20 gestational weeks'	0.07 (-0.03, 0.17)	0.18
Fetal femur length at 20 gestational weeks'	-0.05 (-0.43, 0.34)	0.81

Table 12d: Multiple Linear Regression For Neonatal %FM (with interaction effect)

	β (95% CI)	P-Value
ALL infants		
(Constant)	-6.29 (-23.79, 11.22)	0.48
Female Infant (versus male infant)	2.66 (1.17, 4.15)	0.001
Exclusively Breastfed (versus formula-fed by two months old)	2.55 (1.26, 3.84)	<0.001
Gestational weeks at delivery	0.32 (-0.03, 0.68)	0.08
Maternal occupation is manager/professional	-0.61 (-1.49, 0.28)	0.18
Maternal BMI at 15 weeks' gestation	0.04 (-0.15, 0.22)	0.70
Mother had completed tertiary education (versus not completed tertiary education)	0.13 (-0.79, 1.05)	0.78
Maternal waist (cm) at 15 gestational weeks'	0.008 (-0.08, 0.09)	0.86
Maternal hip (cm) at 15 gestational weeks'	0.01 (-0.03, 0.05)	0.54
Number of cigarettes smoked at 20 gestational weeks'	0.08 (-0.08, 0.24)	0.31
Fetal biparietal diameter at 20 gestational weeks'	0.009 (-0.37, 0.39)	0.96
Fetal head circumference at 20 gestational weeks'	-0.07 (-0.19, 0.06)	0.31
Fetal abdominal circumference at 20 gestational weeks'	0.06 (-0.02, 0.13)	0.17
Fetal femur length at 20 gestational weeks'	0.09 (-0.17, 0.35)	0.51
Sex* Feeding Group* Fetal biparietal diameter at 20 gestational weeks'	-0.47 (-1.16, 0.21)	0.18
Sex* Feeding Group* Fetal head circumference at 20 gestational weeks'	0.10 (-0.12, 0.31)	0.37
Sex* Feeding Group* Fetal abdominal circumference at 20 gestational weeks'	0.03 (-0.12, 0.17)	0.72

Chapter 5: Early life factors associated with the exclusivity and duration of breastfeeding in an Irish birth cohort.

ABSTRACT:

Objective: To investigate the influence of parental and infant characteristics on exclusive breastfeeding from birth to 6 months of age and breastfeeding rates at 2, 6 and 12 months of age in Ireland.

Methodology: Secondary data analysis from the Cork BASELINE Birth Cohort Study (http://www.baselinestudy.net/). Infants were seen at birth and 2, 6, and 12 months of age. Feeding data were collected using parental questionnaires at each time point and exclusive breastfeeding was defined as per the World Health Organisation definitions.

Participants: 1,094 singleton infants of primiparous mothers recruited at 20 weeks' gestation who were breastfeeding on discharge from the maternity hospital.

Findings: Infants who were admitted to the neonatal intensive-care unit (NICU) were less likely to be exclusively breastfed at both discharge from the maternity hospital (adjusted OR 0.17, 95% CI 0.07-0.41) and at 2 months (adjusted OR=0.20, 95% CI 0.05-0.83). The duration (days) spent in the NICU was also negatively associated with exclusive breastfeeding at 2 months. There was a significant difference in the duration of any breastfeeding between infants who were and were not admitted to the NICU, 28 (10.50, 32) weeks vs 32 (27, 40) weeks. Mothers whose maternity leave was between 7-12 months (adjusted OR=2.76, 95% CI 1.51-5.05) breastfed for a longer duration compared to mothers who had less than 6 months of maternity leave.

Key conclusions: Admission to the neonatal intensive care unit negatively influenced both exclusivity and duration of breastfeeding. Length of maternity

leave, and not employment status, was significantly associated with duration of breastfeeding.

Implications for practice: Although admission to the NICU may not be modifiable the breastfeeding support available to mothers is and this requires evaluation. Length of maternity leave is a modifiable influence on breastfeeding and offers the opportunity for intervention to improve our rates of breastfeeding.

Introduction:

In 2003 Ireland adopted the World Health Organization (WHO) recommendations for exclusive breastfeeding in the first 6 months of life (Department of Health & Children, 2003). Recently there has been an increase in breastfeeding initiation rates in Irish maternity hospitals (Health Research and Information Division, 2013) but this has not translated into increased duration of breastfeeding for either “exclusive” or “any” breastfeeding. Ireland continues to have one of the lowest breastfeeding rates internationally (The Economic and Social Research Institute, 2012).

Breastfeeding is an intricate health behaviour and studies have shown that the reasons and influences for mothers to breastfeed are multi-factorial and complex. Empirical research has found postnatal depression, anxiety, social/paternal support to be among factors influencing the duration of exclusive breastfeeding. Overall, maternal self-efficacy was the most reported psychosocial factor and it was found to be the most effective in influencing exclusive breastfeeding duration (De Jager et al., 2013).

Internationally, demographic factors, such as maternal age, smoking, employment and education level, marital status and household income have all been found to significantly influence breastfeeding duration (Dennis et al., 2013). There is limited research focusing on the predictors of exclusive breastfeeding and studies that have examined exclusive breastfeeding have found conflicting results (Chudasama et al., 2008; Dennis et al., 2013;

Dubois and Girard, 2003; Jones et al., 2011; De Jager et al., 2013; Kristiansen et al., 2010; Scott et al., 2006; Tan, 2011; Vieira et al., 2014). Hospital practices have also been shown to influence breastfeeding rates (Giovannini et al., 2005; Merten et al., 2005). The critical role that maternity services provide in promoting and supporting breastfeeding has been recognised internationally (World Health Organization and UNICEF, 1989). In 1991 the WHO and UNICEF developed the Baby Friendly Hospital Initiative (BFHI) in an effort to promote best breastfeeding practices in hospitals to protect and strengthen breastfeeding rates (World Health Organization, 1998).

Studies specific to the Irish breastfeeding population have examined which factors are associated with any breastfeeding (Tarrant et al., 2011b; Leahy-Warren et al., 2014) but not exclusive and few have descriptively described which mothers were exclusively breastfeeding (Begley et al.,2008; Williams et al., 2010).

We wished to examine the early life factors which affected duration of breastfeeding and exclusivity of breastfeeding in a prospective maternal-infant cohort.

Methods

Design and setting

All infants were born in a single large maternity hospital, which has a BFHI certificate of membership. A certificate of membership is awarded to hospitals that are participating in the initiative but do not yet meet the criteria for BFHI accreditation.

Data collection

Gestational age at delivery was classified as preterm ($\leq 36^{+6}$gestational weeks) or term ($\geq 37^{+0}$gestational weeks). Maternal socio-economic status (SES) was determined using the Irish Central Statistical Office (CSO) guidelines (Central Statistics Office, 2006). Infants were categorised as exclusively breastfed per WHO definitions if they received breast-milk only (with the exception of medicines, vitamins, minerals and oral rehydration solution). Partial breastfeeding included infants who had received any artificial feeds and/or solid food (World Health Organization, 2001).

Statistical analyses

Descriptive analysis was undertaken to determine the prevalence of exclusive breastfeeding and rates of breastfeeding at each time point. Breastfeeding duration was tested using Mann-Whitney test when examining between two categories and Kruskal-Wallis test was employed when investigating across three or more categories.

Significant factors, identified through univariate analysis, were entered into logistic regression models to assess for the adjusted odds ratio (adjusted OR) for exclusive breastfeeding at discharge from the maternity hospital and at 2 and 6 months and on breastfeeding rates at 2, 6 and 12 months of age.

Findings

At 20 weeks' gestation 1,537 mothers consented for their child to participate in the Cork BASELINE Birth Cohort Study and 1,094 (71.18%) were exclusively or partially breastfeeding their infant on discharge from the maternity hospital. The response rate of breastfeeding mothers at 2, 6 and 12 months was 999 (91.3%), 966 (88.3%) and 909 (83.1%), respectively. In total, 874 (79.9%) of breastfeeding mothers attended all three appointments with their infants. There was no evidence that mothers who did not respond at follow-ups differed from those who did in any important respects.

The majority of mothers were married (70.9%) or co-habiting with their partner (20.6%) and 58.0% had a university (degree or higher) qualification. A minority (5.7%) of mothers reported smoking during their pregnancy. Over half of the infants were delivered vaginally (unassisted 37.4% and instrumental 37.1%). Mean(SD) gestational age was 39.94(1.66) weeks and average birth weight was 3.47(0.53)kg.

In total 12.7% of the cohort were admitted to the NICU, see Table 13. Respiratory distress (32.4%), requiring phototherapy (28.1%) and other (25.2%) were the three most common reasons reported for admission to the NICU. Other reasons listed included, in order of frequency: infection, preterm delivery, feeding problems, hypoglycaemia, cyanosis, birth asphyxia, small-for-gestational age and congenital abnormality.

Hospital records showed that at discharge 469 (42.9%) of infants left the maternity hospital exclusively breastfeeding and the remaining 625 (57.1%) breastfed infants had received formula supplementation at least once prior to discharge.

Exclusive Breastfeeding at discharge:

In univariate analysis factors associated with exclusive breastfeeding at discharge included: normal maternal and paternal BMI status, unassisted vaginal delivery of a term infant who was not admitted to the NICU, infant birth-weight between 2.51-3.99kg and staying four days or less in the maternity hospital following delivery.

Once all significant factors were entered into logistic regression, paternal BMI, preterm delivery and birth weight groups no longer significantly influenced exclusive breastfeeding. Admission to the NICU had the strongest influence on a mother not exclusively breastfeeding her infant at discharge from the maternity hospital. The odds of an infant, who was admitted to the NICU, being exclusively breastfed on discharge from the maternity hospital were over five times lower (adjusted OR=0.17, 95% 0.07-0.41) compared to infants who were not admitted to the NICU (Table 14). In total 92.1% of breastfeeding infants admitted to the neonatal unit had been given formula supplementation prior to discharge.

Admission to the NICU was examined further to see if the reason for admission or the length of stay affected the rate of exclusive breastfeeding. None of the listed reasons for admission to the NICU or length (days) of stay in the NICU was not associated with exclusive breastfeeding at discharge.

Exclusive breastfeeding at 2 months:

At 2 months of age maternity care practices (mode of delivery, length of stay in the maternity hospital following delivery and admission to the NICU) continued to be the most commonly associated factors with exclusive breastfeeding.

In adjusting for all identified significant factors, admission to the NICU remained the strongest predictor of exclusive breastfeeding at 2 months of age. The odds of an infant, who was admitted to the NICU, being exclusive breastfed at 2 months were five times lower (adjusted OR=0.20, 95% 0.05-0.83) compared to infants who were not admitted to the NICU (Table 14). Maternal obesity and prolonged length of stay in the maternity hospital (more than 5 days) were also associated with a reduction in exclusively breastfeeding at 2 months (Table 14).

Admission to the NICU was again investigated further to see if the reason for NICU admission or if the NICU length of stay also increased the odds for a mother to have stopped exclusive breastfeeding before 2 months. The reasons for admission which were negatively associated with rates of exclusive breastfeeding at 2 months were birth asphyxia (p=0.020) and infection (p=0.049). Length of stay in the NICU was negatively associated with exclusive breastfeeding at 2 months, (p=0.001).

Exclusive breastfeeding at 6 months:

Our cohort had extremely low rates of exclusive breastfeeding at 6 months 7/909 (0.7%). Older maternal age was the only variable found to be significantly associated with exclusive breastfeeding as per WHO recommendations of six months ($p \leq 0.001$). This may be due to the small sample size of 7 mothers that did exclusively breastfed for 6 months, which limited our ability to investigate for any predictive factors.

Breastfeeding duration

Rates of exclusive breastfeeding dropped by 39.6% in the first 8 weeks of life and only seven mothers reported exclusive breastfeeding for the first six months of life. By 12 months of age 8.0% (n=87) of infants were receiving any breast-milk, no infant was exclusively breastfed at 12 months of age, see Figure 2. Duration of breastfeeding significantly differed between infants who were exclusively breastfed on discharge from the maternity hospital and those that had been supplemented with formula in the postnatal period. Infants that were not exclusively breastfed on discharge were breastfed for a shorter period of time: 30(25.50, 37) weeks vs 32(28, 42) weeks, p=<0.001.

Overall, infants who left the maternity hospital exclusively breastfed had significantly greater odds of being breastfeeding at 2 months (adjusted OR=3.02, 95% CI 2.22-4.10), 6 months (adjusted OR=2.04, 95% CI 1.40-2.98) and 12 months (adjusted OR=2.03, 95% CI 1.12-3.67) compared to infants that left the maternity hospital partially breastfed, see Tables 15, 16 and 17.

Maternal and paternal nationality, maternal tertiary education, preterm delivery and admission to the NICU were all significantly associated with breastfeeding status at each time point.

There was a significant difference in the duration of any breastfeeding between infants who were and were not admitted to the NICU; 28(10.50, 32) weeks vs 32(27, 40) weeks, p=0.019. No significant difference was found between duration of breastfeeding and any of the listed reasons for admission (p=0.250).

The odds of a mother, born outside of Ireland, to be breastfeeding at 2 months (adjusted OR=2.43, 95% CI 1.59-3.73) were twice that of a mother born in Ireland and this trend continued at 6 months (adjusted OR=2.39, 95% CI 1.46-3.91). In maternal age groups, the odds of a mother, aged 19-23 years, to be breastfeeding at 2 months (adjusted OR=0.38, 95% CI 0.17-0.86) were lower compared to mothers aged 30-34 years-old, no other age group significantly influenced breastfeeding at 2 months. Mothers with a tertiary education had nearly twice the odds to be breastfeeding at 2 months compared to mothers with no tertiary education (adjusted OR= 1.88, 95% CI 1.38-2.57), see table 15.

At 6 months maternal tertiary education continued to positively influence breastfeeding rates. Mothers with a tertiary education had greater odds to be breastfeeding compared to mothers with no tertiary education (adjusted OR= 1.63, 95% CI 1.08-2.46). Almost 86% of the mothers in our cohort were in employment during their pregnancy. Within this working group of mothers, we found that maternity leave of 7-12 months (adjusted OR = 2.76, 95% CI 1.51-5.05) was associated with a longer breastfeeding duration compared to maternity leave that was less than 6 months, see Table 16. We investigated the reasons given by 688 (91.01%) out of the 756 mothers, who were in employment and had stopped breastfeeding by the 12 month appointment, for why they had ceased breastfeeding. Returning to work was the most common (n=135, 19.62%) reason for mothers to stop breastfeeding their child.

At 12 months infants born to a mother with a tertiary education (adjusted OR=2.35, 95% CI 1.20-4.62) and infants who had left the maternity hospital exclusively breastfeeding (adjusted OR=2.03, 95% CI 1.12-3.67) had twice the odds of being breastfed compared to infants born to mothers with no tertiary education and those that had received infant formula supplementation in the maternity hospital, respectively (Table 17).

Discussion

We have established which early life factors influenced both exclusive breastfeeding in the first 6 months and non-exclusive breastfeeding rates at 2, 6 and 12 months of age in a cohort of primiparous mothers who initiated breastfeeding. We have identified the key areas surrounding exclusive breastfeeding in the first six months of life and breastfeeding duration in the first year of life. Our results are important to both the Irish setting and for other countries experiencing an increase in breastfeeding initiation but have not seen an increase in their exclusive breastfeeding rates or the duration of time mothers report breastfeeding their infants (Hamade et al., 2013).

Maternity care practices (mode of delivery, admission to the NICU and duration of stay in the maternity hospital following delivery) were all significantly associated with exclusive breastfeeding at discharge from the maternity hospital and at 2 months of age. Admission to the NICU, and not the reason for admission or length of stay in the NICU, was associated with both decreased rates of exclusive breastfeeding and the duration of any breastfeeding. Lower breastfeeding rates in the NICU compared to the postnatal wards in maternity hospitals has been previously reported (Wallace et al., 2013). Previous studies investigating breastfeeding rates in NICU have primary focused on a specific neonatal population (Lee et al., 2012; Maia et al., 2011; Bonet et al., 2010) and not on a population-based cohort. We have found that admission to the NICU, independent of the reason, including preterm delivery and low birth-weight, negatively impact on breastfeeding. This is a critical finding for maternity services as our results suggest admission to the NICU requires attention to ensure that all mothers receive the necessary and appropriate support to maintain breastfeeding.

While undertaking this PhD and completing this paper I linked in with the Clinical Midwifery Specialist (CMS) for Breastfeeding/ Lactation Consultant for the maternity hospital. As a result of this paper I supported the development of an in-house breastfeeding audit tool, data collection, undertook all data analysis and wrote the report on the rate of formula supplementation among breastfeed infants on the postnatal wards and reasons for the supplementation. This audit highlighted that the need for education around hypoglycaemia management for neonates on the postnatal ward. One area of practice that did change following the publication of this paper in Midwifery is the provision of phototherapy by the bedside. Previously all neonates who required phototherapy were admitted to the NICU.

Previous studies (Nickel et al., 2013; Tarrant et al., 2011a; Declercq et al., 2009; Giovannini et al., 2005; Merten et al., 2005) examining exclusive breastfeeding with maternity care practices have only explored associations between the exposure and outcome but have not investigated the relationship between covariates. Obviously, mode of delivery, admission to NICU and length of hospital stay are all strongly interrelated and were significantly associated with each other in our cohort (p=<0.001). Of these we found that NICU admission had the strongest association, further supporting that management of feeding in NICU should be an area of focus for maternity services to protect and promote breastfeeding.

Tertiary maternal education and exclusive breastfeeding at discharge from the maternity hospital were the only two characteristics that positively influenced breastfeeding rates consistently at 2, 6 and 12 months. Household income and maternal employment status did not influence breastfeeding duration but for mothers who were employed the length of their maternity leave significantly influenced whether they continued to provide any breast-milk to their infant. Data from the national cohort Growing Up in Scotland survey has also demonstrated that it is the length of maternity leave that influences breastfeeding duration and not the employment status of the mother (Skafida, 2012). We found that mothers who were breastfeeding at 2, 6 and 12 months reported longer maternity leave than mothers who stated that they were no longer breastfeeding at 2, 6 and 12 months. Returning to work was the most common reason mothers gave for stopping to breastfeed, although most stated that they breastfed as long as they had planned. This suggests that mothers determine how long they would like to breastfed based on their planned duration of maternity leave. This is supported by results from the United States' Infant Feeding Practices Study II (2005-2007) which found that mothers decided how long they wished to breastfed for based on their duration of maternity leave (Mirkovic et al., 2014).

Maternal non-Irish nationality increased rates of breastfeeding at 2 and 6 months of age and this has been reported in previous Irish studies (Begley et al., 2008; Tarrant et al., 2011b). As the rate of mothers born outside Ireland increases, so too has the rate of breastfeeding initiation increased (Health Research and Information Division, 2013). This is in line with other studies in England and America (Brick and Nolan, 2013). These results suggest that it may be the change in maternal characteristics rather than breastfeeding promotion that is increasing breastfeeding rates (Ladewig et al., 2014).

Our study population are predominantly low-risk healthy professional mothers with a tertiary education, in current employment and non-smokers. All infants are singleton and most were born at term without medical complications. Previous research would indicate that this population would be the most highly motivated to continue breastfeeding their infant after hospital discharge (Ekström et al., 2003). In examining this population of breastfeeding mothers we have found that the characteristics involved in exclusive breasting differed to what influenced breastfeeding duration and the effect of various characteristics on breastfeeding duration changed over time. These are important findings which we hope will inform effective breastfeeding promotion and support (Simard et al., 2005; Thulier and Mercer, 2009).

The terms 'fully' and 'exclusive' are often used interchangeable (Scott et al., 2006; Chudasama et al., 2008; De Jager et al., 2013) making it difficult to determine if the study population are actually being exclusively breastfed. Few studies have accurate prospective data available on the immediate maternity and neonatal care provided to the infant and their mother (Giovannini et al., 2005; Jones et al., 2011; Tan, 2011). There are limitations to our study. This is a secondary data analysis; investigating breastfeeding practice was not a primary objective of the Cork BASELINE Birth Cohort Study. Breastfeeding is a complex health behaviour, driven by many connecting factors (Scott et al., 2006; Semenic et al., 2008). Our study did not collect data on other hospital practices (such as rooming-in, skin-to-skin contact) or contact with other healthcare professionals, psychological or social factors. Hospital practices, interactions with other healthcare professionals outside of the maternity setting, feeding intentions, societal and family support for breastfeeding are influential in how a mother feeds her child (Difrisco et al., 2011; Whelan et al., 2011; Odom et al., 2014; Shortt et al., 2013). Strengths of this paper include the consistent use of WHO definitions to describe breastfeeding patterns (Fewtrell, 2011) and, in comparison to others studies (Giovannini et al., 2005; Jones et al., 2011; Tan, 2011), we also prospective collected information on maternity care practices. Our study has identified two key areas in both maternity care practices and national policy on maternity leave that have potential to convert breastfeeding initiation into increased duration of breastfeeding.

Key conclusion

This study sought to examine what parental and infant characteristics influenced exclusive and any breastfeeding in a country with increasing breastfeeding initiation rates but not an overall increase in breastfeeding rates. We have found that admission to the NICU significantly reduced the odds of an infant being exclusively breastfed on discharge from the maternity hospital and at 2 months and that admission to the NICU also significantly reduced the duration of any breastfeeding. Admission to the NICU is potentially a time of focussed intervention and maternity services need to ensure that they are effectively supporting breastfeeding mothers whose infants have been admitted to the NICU.

We also found that maternity leave, independent of SES, has a significant effect on breastfeeding duration. Countries that provide mothers with paid maternity leave greater than six months also report some of the world’s highest breastfeeding (exclusive and duration) rates (Save The Children, 2012). Countries with low breastfeeding rates should re-evaluate their current maternity leave provision, and in work facilities for breastfeeding mothers which may help to improve their current breastfeeding rates.

Table 13: Baseline characteristics of study participants

	N	%		N	%		N	%
Infant sex			**Maternal BMI**			**Maternal Nationality**		
Male	546	49.9	Normal (18.5-24.99)	671	61.3	Born in Ireland	809	73.9
Female	548	50.1	Underweight (<18.5)	12	1.1	Born Outside of Ireland	244	20.5
Admission to neonatal unit			Overweight (25-29.99)	298	27.2	**Paternal BMI**		
No	955	87.3	Obese (30-40.50)	110	10.1	Normal (18.5-24.99)	267	24.4
Yes	139	12.7	**Maternal employment**			Underweight (<18.5)	1	0.1
Maternal age			Unemployed	96	8.8	Overweight (25-29.99)	455	41.6
19-23 years	49	4.5	Employed	937	85.6	Obese (30-40.50)	115	10.5
24-28 years	191	17.5	Missing	61	5.6			
29-33 years	586	53.6	**Maternity leave**					
34-38 years	225	20.6	<6 months	95	8.7			
39-43 years	42	3.8	6 months	123	11.2			
44-48 years	1	0.09	7-12 months	535	48.9			
Maternal tertiary education			>12 months	1	0.1			
No	399	36.5	Not applicable	96	8.8			
Yes	634	58.0	Missing	244	22.3			

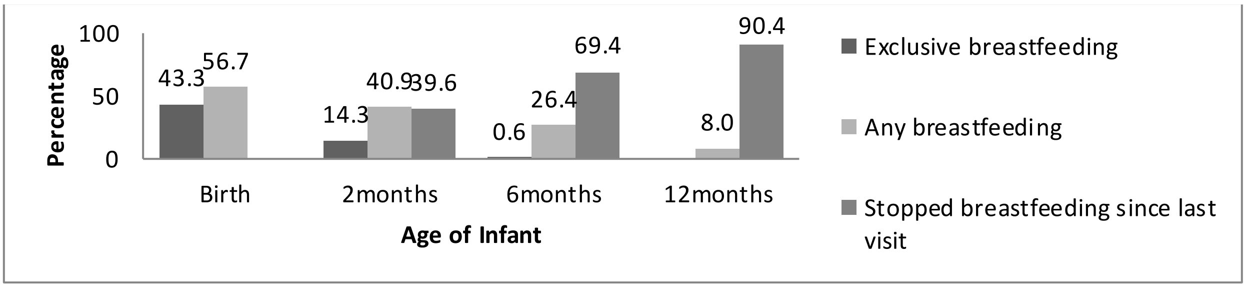


Figure 2: Rates of exclusive, any or stopped breastfeeding at birth and 2, 6 and 12 for infants breastfed in the maternity hospital

Table 14: Adjusted odds ratios for exclusive breastfeeding at birth (n= 834) and 2 months (n=601)

	Birth	**2 months**
	Adjusted OR (95% CI)	Adjusted OR (95% CI)
Gestational age at delivery		
Preterm (≤36w 6d)	0.26 (0.06-1.12)	
Term (≥37w 0d)	1	
Mode of delivery		
Unassisted vaginal delivery	1	1
Assisted vaginal delivery	0.70* (0.49-0.98)	0.66 (0.42-1.02)
Elective caesarean section	1.40 (0.69-2.87)	2.56 (1.00-6.56)
Emergency caesarean section	0.80 (0.41-1.57)	1.08 (0.41-2.83)
Birth-weight groups		
≤2.50kg	1.90 (0.35-10.29)	
2.51-3.99kg	1	
≥4.kg	0.98 (0.62-1.53)	
Admission to neonatal unit		
No	1	1
Yes	0.17 (0.07-0.41)***	0.20 (0.05-0.83)**
Duration of stay in the maternity hospital		
1 day	1	1
2 days	0.44 (0.18-1.04)	1.82 (0.66-5.00)
3 days	0.42 (0.18-1.01)	1.09 (0.39-2.98)
4 days	0.17 (0.06-0.47)**	0.49 (0.14-1.71)
5 days	0.09 (0.03-0.30)***	0.17 (0.31-0.92)*
6 days	0.52 (0.01-0.51)*	0.00 (0.00)
≥1 week	0.27 (0.40-1.80)	1.07 (0.12-9.41)
Maternal BMI		
Underweight (<18.5)	3.11 (0.73-13.24)	0.42 (0.90-19.27)
Normal (18.5-24.99)	1	1
Overweight (25-29.99)	0.86 (0.61-1.21)	0.74 (0.47-1.17)
Obese (30-40.50)	0.40 (0.23-0.69)**	0.34 (0.13-0.85)**
Paternal BMI		
Normal (18.5-24.99)	1	
Overweight (25-29.99)	0.75 (0.56-1.09)	
Obese (30-40.50)	0.65 (0.39-1.07)	

*p≤0.005; **p≤0.01; ***p≤0.001

Table 15: Adjusted odds ratios for any breastfeeding at 2 months (n=992)

	2 months
	Adjusted OR (95% CI)
Gestational age at delivery: Term (≥37w 0d)	1
Preterm (≤36w 6d)	0.48 (0.20-1.12)
Birth-weight groups: 2.51-3.99kg	1
≤2.50kg	0.7 (0.27-1.86)
≥4.kg	1.2 (0.79-1.81)
Admission to neonatal intensive care unit: No	1
Yes	1.1 (0.69-1.77)
Exclusive Breastfeeding on discharge from hospital: No	1
Yes	3.01 (2.22-4.10)***
Smoked during pregnancy: No	1
Yes	0.40 (0.20-0.71)**
Maternal age (years): 29-33	1
19-23	0.38 (0.17-0.86)*
24-28	0.66 (0.44-1.00)
34-38	0.8 (0.56-1.14)
39-43	1.00 (0.47-2.12)
Marital Status: Married	1
Single or Separated	0.98 (0.67-1.44)
Defacto (in a stable relationship but not married)	0.91 (0.37-2.27)
Maternal tertiary education: No	1
Yes	1.88 (1.38-2.57)***
Socio-Economic Status: Professional	1
Managerial and Technical	0.94 (0.65-1.36)
Non-manual	0.73 (0.45-1.17)
Skilled	1.17 (0.54-2.57)
Un-skilled	0.87 (0.38-2.03)
Ungainful and unknown	0.87 (0.42-1.81)
Maternal Nationality: Born in Ireland	1
Born outside of Ireland	2.43 (1.59-3.73)***
Maternal BMI: Normal (18.5-24.99)	1
Underweight (<18.5)	0.98 (0.23-4.19)
Overweight (25-29.99)	0.77 (0.56-1.06)
Obese (30-40.50)	0.68 (0.42-1.11)
Paternal Nationality: Born in Ireland	1
Born outside of Ireland	1.46 (0.95-2.23)

*p≤0.005; **p≤0.01; ***p≤0.001

Table 16: Adjusted odds ratios for any breastfeeding at 6 months (n=722)

	6 months
	Adjusted OR (95% CI)
Gestational age at delivery: Term (≥37w 0d)	1
Preterm (≤36w 6d)	0.19 (0.34-1.10)
Birth-weight groups: 2.51-3.99kg	1
≤2.50kg	0.51 (0.04-6.62)
≥4.kg	0.82 (0.49-1.38)
Admission to neonatal intensive care unit: No	1
Yes	0.47 (0.22-0.98)*
Exclusive Breastfeeding on discharge from hospital: No	1
Yes	2.04 (1.40-2.98)***
Duration of stay in the maternity hospital: 1 day	1
2 days	1.24 (0.45-3.42)
3 days	1.48 (0.54-4.02)
4 days	1.30 (0.46-3.66)
5 days	1.77 (0.53-5.89)
6 days	5.61 (1.08-29.02)*
≥1 week	1.46 (0.24-8.97)
Smoked during pregnancy: No	1
Yes	0.35 (0.11-1.12)
Maternal tertiary education: No	1
Yes	1.63 (1.08-2.46)*
Duration of maternity leave:<6 months	1
6 months	0.93 (0.44-1.97)
7-12 months	2.76 (1.51-5.05)**
Socio-Economic Status: Professional	1
Managerial and Technical	0.58 (0.38-0.89)*
Non-manual	0.47 (0.25-0.87)*
Skilled	1.06 (0.40-2.83)
Un-skilled	0.30 (0.85-1.03)
Ungainful and unknown	0.75 (0.23-2.51)
Maternal Nationality: Born in Ireland	1
Born outside of Ireland	2.39 (1.46-3.91)**
Maternal BMI: Normal (18.5-24.99)	1
Underweight (<18.5)	1.71 (0.31-9.43)
Overweight (25-29.99)	0.67 (0.44-1.02)
Obese (30-40.50)	0.26 (0.11-0.61)*
Paternal Nationality: Born in Ireland	1
Born outside of Ireland	2.52 (1.51-4.20)***

*p≤0.005; **p≤0.01; ***p≤0.001

Table 17: Adjusted odds ratios for any breastfeeding at 12 months (n=704)

	12 months
	Adjusted OR (95% CI)
Gestational age at delivery: Term (≥37w 0d)	1
Preterm (≤36w 6d)	0 (0.00-0.00)
Infant Sex: Male	1
Female	1.54 (0.87-2.72)
Admission to neonatal intensive care unit: No	1
Yes	0.14 (0.02 - 1.10)
Exclusive Breastfeeding on discharge from hospital: No	1
Yes	2.03 (1.12-3.67)*
Maternity Care: Public	1
Private	0.48 (0.23-0.99)*
Marital Status: Married	1
Single or Separated	0.30 (0.12-0.76)*
Defacto (in a stable relationship but not married)	4.40 (0.66-29.41)
Maternal tertiary education: No	1
Yes	2.35 (1.20-4.62)*
Maternal employment: Unemployed	1
Employed	0.46 (0.20-1.07)
Maternal Nationality: Born in Ireland	1
Born outside of Ireland	1.61 (0.83-3.12)
Paternal Nationality: Born in Ireland	1
Born outside of Ireland	2.27 (1.17-4.41)**
Paternal BMI: Normal (18.5-24.99)	1
Overweight (25-29.99)	0.70 (0.37-1.31)
Obese (30-40.50)	1.82 (0.85-3.89)

*p≤0.005; **p≤0.01; ***p≤0.001

Chapter 6: Infant formula feeding practices in a prospective population-based birth cohort study

ABSTRACT

Background: It is recommended that formula-fed infants are given standard whey-based infant formula throughout the first year of life, unless otherwise advised by healthcare professionals. To our knowledge it has not yet been explored if parents are using a whey-based infant formula throughout the first 12 months of life. Reasons for parental choice of formula are also unknown. Therefore, the objective of this paper was to describe parental administration of whey-based and non whey-based infant formula in the first year of life.

Methods: Data collected as part of the Cork BASELINE Birth Cohort Study examined infant feeding practices at 2, 6 and 12 months of age. Descriptive analysis explored infant feeding practices and parental reasons for changing from a whey-based to a non whey-based infant formula. Multiple logistic regression investigated parental and infant characteristics associated with the use of whey-based infant formula.

Results: In total, 62.4%, 40.4% and 12.8% parent(s) at 2, 6 and 12 months, respectively, gave their infant whey-based infant formula. No parental or infant characteristic was found to consistently influence the use of whey-based infant formula. The most common reason reported by parent(s) for changing their infant's formula to a non whey-based formula was that they perceived their baby as being hungry.

Conclusion: The majority of parent(s) commence their infants on whey-based formula, but most change to non whey-based formula before 12 months of age. Parental perception of infant satiety and not healthcare advice was the most common reason for changing from a whey-based to a non whey-based infant formula. Additional research is now required to investigate the effect of whey-based and non whey-based infant formula on infant growth.

Background

Breastfeeding is the internationally recommended method of infant feeding[207] with proven benefits over infant formula feeding[208]. For infants who are not breastfed the Baby Friendly Initiative (BFI), (a programme supported by the World Health Organization and United Nations Children's Emergency Fund), recommend the use of a standard whey-based infant formula in the first 12 months of life, unless medically indicated by a healthcare professional[6, 391]. This recommendation is supported by Food Safety Authority of Ireland[67, 392]. There are different categories of infant formula and for an infant formula to be described as a standard whey-based infant formula it needs to have a whey:casein protein ratio of 60:40[393]. Only infant formula labelled as 'newborn' or 'first milk' meet this definition. Therefore, the majority of infant formula (i.e. soya-based, hydrolysed, follow-on or growing-up infant formula) can be categorised as non whey-based infant formula. To our knowledge it remains unknown what types of infant formula are being used in the first year of life.

The World Health Organization (WHO) recommend that infant feeding practices are regularly monitored[394] but the literature on type of infant formula practices, in comparison to breastfeeding practices, is scant[143, 155, 156, 158-160]. To our knowledge only one study[160] reported the use of standard whey-based infant formula at six weeks postpartum but did not examine the use of standard whey-based infant formula throughout the first year of life.

Therefore, in a population-based birth cohort, we wished to describe both the use of whey-based and non whey-based infant formula during the first 12 months of life and parental self-reported reasons for infant formula changes. We also examined what parental and infant characteristics were associated with the use of whey-based infant formula.

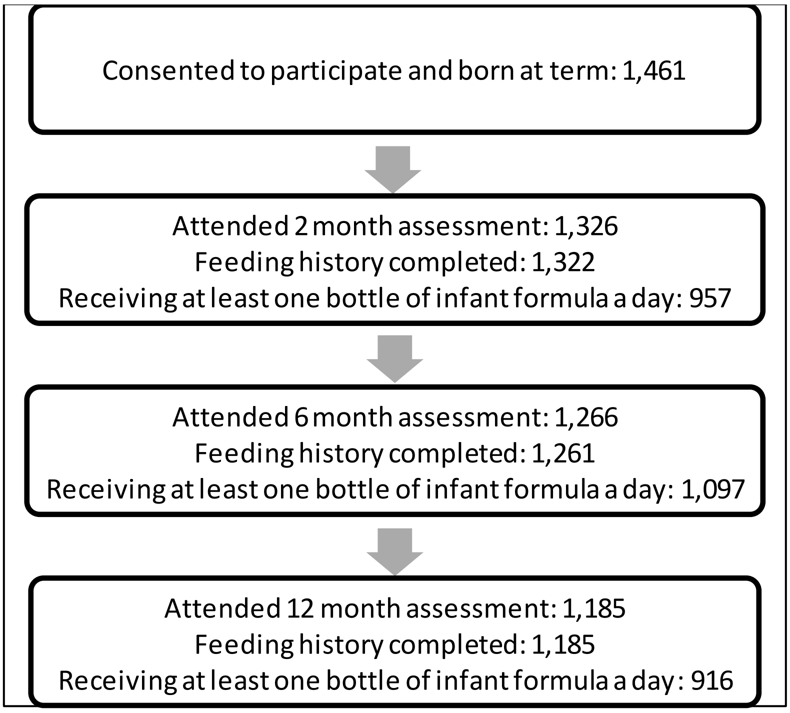
Methods:

Descriptive analysis examined infant feeding practices and reasons for using a non whey-based infant formula. Each time point was examined independent of each other.

Results:

From the SCOPE Ireland Study 1,461 mothers, who delivered a term infant, had consented to continue with the BASELINE Birth Cohort Study. Within this cohort 99 parents did not return with their infants for any of the follow-up visits and 71 parents reported that their infant was not given infant formula at least once a day at any of the follow-up appointments. This left, in total, 1291 (88.4%) infants available for analysis (see Figure 3). We examined for, and found no evidence, of attrition bias between any of the time points. Reasons for parents withdrawing their infant from the study included time and travel constraints.

Figure 3: Participant flowchart



* In total, from all three follow-up appointments, 1291 infants were included for analysis

Nearly all (94.9%) mothers were married or in a stable relationship. The overall mean(SD) birthweight was 3.51(0.45)kg and the admission rate to the neonatal unit (NNU) was 9.4%, (Table 18).

Table 18: Characteristics of study population

Characteristic of study population (n = 1,291)	**N (%) or mean(SD)**
Infant Sex	
Male	653 (50.6%)
Female	638 (49.4%)
Gestational age (weeks) at delivery	40.17(1.14)
Birth-weight (kg)	3.51(0.46)
Maternal Age (years)	31.11(4.36)
Maternal Nationality	
Irish	1078 (83.5%)
Non-Irish	213 (16.5%)
Paternal Nationality[*]	
Irish	1093 (84.7%)
Non-Irish	193 (14.9%)
Mother reported smoking during pregnancy[*]	
No	1132 (87.7%)
Yes	119 (9.2%)
Maternal Tertiary Education	
No	613 (47.5%)
Yes	678 (52.5%)
Maternal Employment Status	
Unemployed	126 (9.8%)
Employed	1165 (90.2%)
Maternity Care	
Public	938 (72.7%)
Private	353 (27.3%)

[*] Percentages do not equal 100 as some mothers did not answer the question

Parental use of whey-based infant formula decreased as the infants got older. At 2 months 62.4% of mothers reported giving their infant a whey-based infant formula. This figure dropped to 40.4% and 12.8% at 6 and 12 months, respectively.

At 2 months the two most popular non whey-based infant formula were those marketed as ‘suitable for hungrier babies’ followed by ‘comfort’ infant formula. This is reflected by the reasons reported by parent(s) for selecting a non whey-based infant formula. The most common reason was that they perceived their infant to be hungry (34.0%) followed by that they didn’t think the current formula suited their infant (17.8%) or the advice of health professionals (12.2%), (Table 19).

Table 19: Overall, the most common reported parental reasons for changing infant formula

Reported reason	2 months N (%)	6 months N (%)	12 months N (%)
Infant was hungry	122 (34.0%)	202 (30.8%)	60 (7.5%)
Advice of healthcare professional	44 (12.2%)	35 (5.3%)	35 (4.4%)
Followed label guidelines	1 (0.3%)	157 (24.0%)	249 (31.2%)

Parental use of ‘follow-on’ infant formula (n=728) and ‘Growing-up milk’ (n=262) were the main reasons for the decreased use of whey-based infant formula at 6 and 12 months. Follow-on infant formula is promoted by infant formula companies as suitable from 6 months of age to complement the period when infants are weaned on to solid food and ‘Growing-up milk’ is promoted as suitable for children from one year of age. Both are considered to be non whey-based infant formula.

At 6 months the most common reasons reported by parents for selecting a non whey-based infant formula were parental perception of a lack of infant satiety

(30.8%) followed by parent(s) saying that they followed the label advice on the infant formula containers (24.0%), parent(s) reporting that their infant was suffering with reflux (7.0%) and the advice of health professionals (5.3%). At 12 months following label guidelines was the most common (31.2%) reason reported by parent(s) for using a non-whey based infant formula, followed by their perception that their infant was hungry (7.5%) and the advice of a healthcare professional (4.4%), (Table 19).

In investigating, through univariate analysis, which factors are associated with using a whey-based infant formula no maternal, paternal or infant characteristics was consistently associated with using a whey-based infant formula across the three time points; 2, 6 and 12 months. Method of feeding at hospital discharge and maternal tertiary education were associated with using a whey-based infant formula at 2 and 6 months. Maternal nationality was associated with this choice at 2 and 12 months. At 6 months infant sex, maternal age and employment status and maternity care were all significantly associated with infants being fed a whey-based infant formula.

In the multivariable analysis, infants that were formula fed on discharge from the maternity hospital had less odds of having a standard whey-based infant formula at 2 months of age compared to infants that left the maternity hospital exclusively breastfeeding but had since introduced infant formula (aOR=0.54, 95% CI 0.37-0.79), (Table 20).

Table 20: Adjusted odds ratio for using whey-based infant formula at 2 months

N = 953	**aOR (95% CI)**
Method of feeding at hospital discharge	
Exclusive Breastfeeding	Reference
Breastfeeding and formula feeding	0.86 (0.59- 1.24)
Formula feeding	0.54 (0.37-0.79)*
Maternal Nationality	
Mother born outside of Ireland	Reference
Mother born in Ireland	0.83 (0.53-1.31)
Paternal Nationality	
Father born outside of Ireland	Reference
Father born in Ireland	0.71 (0.44-1.13)
Maternal tertiary education	
No tertiary education	Reference
Tertiary education	1.28 (0.97-1.68)

*$p \leq 0.05$

This trend reversed at 6 months and infants that left the maternity hospital both breastfeeding and formula feeding (aOR=1.46, 95% CI 1.05-2.03) or were exclusively formula-fed (aOR=1.52, 95% CI 1.13-2.06) had significantly more odds of receiving a standard whey-based infant formula compared to infants that left the maternity hospital exclusively breastfeeding, (Table 21). We investigated this finding more, to explore why the direction of effect of method of feeding at discharge would differ between the two time points. We found that infants who were exclusively breastfed at discharge from the maternity hospital were more likely to use a whey-based formula at 2 months and then change to a follow-on formula (non whey-based infant formula) at 6 months. Infants that were receiving formula at discharge from the maternity hospital and were given a non whey-based infant formula at 2 months and changed back to a whey-based infant formula at 6 months were changed to a different brand to their first whey-based infant formula.

Table 21: Adjusted odds ratio for using whey-based infant formula at 6 months

N =1,095	**aOR (95% CI)**
Infant Sex	
Male	Reference
Female	0.73 (0.57-0.93)*
Maternity Care	
Public	Reference
Private	1.37 (1.04-1.81)*
Method of feeding at hospital discharge	
Exclusive Breastfeeding	Reference
Breastfeeding and formula feeding	1.46 (1.05-2.03)*
Formula feeding	1.52 (1.13-2.06)*
Maternal Age (years)	1.01 (0.98-1.04)
Maternal tertiary education	
No tertiary education	Reference
Tertiary education	1.11 (0.86-1.45)
Maternal employment status	
Unemployed	Reference
Employed	1.57 (0.97-2.54)

*p≤0.05

Maternal smoking during pregnancy was the only characteristic that was significantly associated with an infant formula choice at 12 months. Mothers that smoked had reduced odds of giving their infant a whey-based infant formula compared to non-smoking mothers (aOR=0.29, 95% CI 0.09-0.93), (Table 22).

Table 22: Adjusted odds ratio for using whey-based infant formula at 12 months

N =916	**aOR (95% CI)**
Maternal smoking status during pregnancy	
Non-smoker	Reference
Smoker	0.29 (0.09-0.93)*
Maternal Nationality	
Mother born outside of Ireland	Reference
Mother born in Ireland	1.91 (1.00-3.67)

*P≤0.005

Discussion:

We have shown that the majority of formula-fed infants are not given a whey-based infant formula for the duration of the first year of life. Use of a whey-based infant formula steadily dropped throughout the first year of life, with 40% of formula-fed infants already on a non whey-based infant formula at 2 months of age.

No overall paternal or infant characteristic appeared to influence the use of a whey-based infant formula throughout the first 12 months. How mothers were feeding their infant at discharge from the maternity hospital was the only characteristic associated with the type of infant formula used at both 2 and 6 months. Infants that were exclusively breastfeeding at discharge from the maternity hospital were more likely to be placed on a follow-on formula at 6 months and growing-up milk at 12 months compared to infants that left the maternity hospital either breastfeeding with infant formula or exclusively formula-fed. In comparison infants that left the maternity hospital receiving formula were more likely to be given a non whey-based infant formula that was designed for unsettled babies or babies suffering from colic, reflux etc.

Given the effect of initial breastfeeding, or not, on the use of whey-based infant formula we explored our findings further. Research has shown that there are differences between mothers who breastfed to those that formula-fed[395-397]. Among these factors maternal education has frequently been shown to influence the type of diet children are given[398]. This was also true in our cohort; rates of maternal tertiary education were over four times higher in mothers who initial exclusively breastfed to mothers who did not. Mothers who initially breastfed exclusively but used a follow-on (non whey-based) infant formula at 6 months reported that they were ‘following label guidelines’ and thought that they ‘had to change’ due to their child’s age. These mothers appeared to be seeking for information on infant formula and relied on the advice provided to them by infant formula manufacturers. In comparison, most mothers who formula-fed from birth and switched between whey-based and non whey-based infant formula reported they did so because they felt that the previous infant formula did not suit their infant or their infant did not like the taste of the formula.

Our findings also suggest that parents are not distinguishing between type and brand of infant formula. Some infants who experienced infant formula changes were placed on the same type of infant formula but were given a different brand.

All infants were delivered in the one maternity setting which currently holds a Breast Feeding Hospital Initiative (BFHI) certificate of commitment. This certificate is awarded to settings who currently do not hold BFHI status but have declared their intention to work towards achieving BFHI accreditation. The BFHI requires that mothers who chose to give their child any infant formula are taught, individually, about formula preparation, handling, storage and feeding but does not include educating parents on current recommendations on type of infant formula[399]. As UNICEF supports the use of a whey-based infant formula when breastmilk is not available[6] this study would suggest that an evaluation of current standards for parental education should encompass all aspects of infant formula feeding, including what type of infant formula to use.

The few studies that have examined infant formula feeding practices have mostly focussed on frequency of formula changes rather than formula constituents. Early studies, carried out in 1980[155] and 1995-1996[156], explored changing from a 'standard' to a 'special' formula but as these studies did not define their groups it is difficult to evaluate their findings. An Israeli study undertaken in four maternal and child health care centres between 2002-2003 found that 47% of infants experienced a formula change in the first six months of life. Most of the formula changes were to another cow's milk based formula (not defined) and, on average, the first change occurred at three months[158]. The EDEN (Étude des Déterminants pré et postnatals du développement et de la santé de l'Enfant) mother-child cohort reported on the effect of the predominant choice of infant formula in the first four months of life on infant growth. The study found that 26% of infants had experienced two or more formula changes in the first four months of life. No significant relationship was found between growth and predominant formula (predominant infant formula was a mixture of whey-based and non whey-based infant formula)[159].

One Irish study did descriptively report the type of infant formula used by parent(s) 6 weeks following delivery[160]. The study involved term ($\geq 37^{+0}$ weeks' gestation) singleton infants born with a birthweight of 2.5kg or greater. Out of the total sample of 450 infants, 368 (81.8%) infants were formula-fed at 6 weeks of age and just over half (n=197; 53.6%) were being given a standard whey-based infant formula. Nearly half (n=181, 49.2%) of all infants had experienced at least one formula change. For infants whose formula was changed, either to a whey-based or non whey-based infant formula, parental reports of their infant's increased hunger/ feeding frequency of 2-3 hours was the most (54.8%) reported reason. The study did not provide any information on the initial type of infant formula, or feeding history on type of infant formula after 6 weeks of age.

An analysis of data from the Infant Feeding Practices Study II (IFPS II) examined the effect of marketing, direct or through health professionals, on formula changes[143]. The authors reported that formula changes were made for mainly non-health reasons (health reason was defined based on stool characteristic or diarrhoea, vomiting and fussiness). In our study, parental perception of their infant’s appetite was the most reported reason for changing infant formula. This was followed by advice the mother had received from a healthcare professional (nurse or doctor) at either a routine appointment (such as vaccination or developmental assessment) or if the mother specifically requested to see a doctor over a concern with her child. The influence of healthcare professionals on formula feeding practices was mainly observed at 2 months of age. At 6 and 12 months more mothers reported that they followed the label guidelines on the infant formula containers or from the helpline of the infant formula company than advice from a healthcare professional in their reason for changing their infant’s formula. This brings to attention the influence of marketing from infant formula companies on changing infant formula.

There are limitations to this study as we did not collect information on parental or healthcare professional knowledge on infant formula feeding guidelines. It therefore remains unknown if parents and healthcare professionals are aware of international guidelines on type of infant formula. We have, however, reported which formula, based on BFI guidelines, infants are exposed to in the first year of life. The WHO recommend that all infant feeding (breast- or formula-feeding) is monitored and this paper addresses the current gap in our knowledge on formula-feeding practices. Our results show that parental reports of infant satiety and marketing from infant formula companies but not advice from healthcare professionals influenced their decision on what type of infant formula to purchase. This research now needs to be followed-up by examining infant health outcomes of infants who received whey-based or non whey-based infant formula.

CONCLUSION: We found that most formula-fed infants are given a non whey-based infant formula in the first year of life. The effect of this feeding practice on infant health is unknown. Further research needs to be undertaken to evaluate the appropriateness and value of current guidelines on type of infant formula. It also needs to be investigated what knowledge health care professionals and parent(s) have on the current guidelines on type of infant formula.

Smith, H. A. 2018. Early milk diet of infants and the effect on their body composition and growth and development in the first two years of life. PhD Thesis, University College Cork.

Chapter 8: Discussion

This PhD set to explore the milk (breastmilk and infant formula) feeding habits of children, and associated characteristics, in their first year of life. This PhD also aimed to examine the effect of an infant's milk diet, at two months of age, on their body composition in the first two months of life, growth in the first two years of life while also examining the influence of their diet and growth on their neurodevelopmental outcomes at 24 months. Finally, this PhD also investigated for differences between the breastfeeding and formula-feeding infants at birth, to determine if any differences exist prior to the exposure of interest.

Results from this PhD show that over half of all breastfeeding infants received infant formula supplementation at least once during their stay in the maternity hospital following delivery. Formula-supplementation in the maternity hospital had a significantly negative effect on duration of any breastfeeding at all three time points; two, six and 12 months of age. Admission to the NICU, but not reason for admission, also reduced the odds of exclusive breastfeeding at discharge from the maternity hospital and at two months of age and any breastfeeding at six months of age. These findings highlight the importance of supporting exclusive breastfeeding in maternity hospitals, for all infants (admitted or not admitted to the NICU), to support breastfeeding duration in the first year of life. Another important finding was that for mothers in employment, independent of SES, their length of maternity leave influenced the duration of breastfeeding. Mothers with a maternity leave of seven to 12 months had nearly triple the odds of continuing to breastfed at six months compared to mothers with a maternity leave less than six months. In examining the qualitative data working mothers stated they wanted their children 'settled' on bottles before they went to work and planned their duration of breastfeeding based on their length of maternity leave. This is an important finding, as working mothers stated they had breastfed for as long as they wanted but the period that they wanted to breastfed for was as long as they were on maternity leave. It also highlights that mothers in Ireland are not continuing to provide breastmilk to their children once they return to work.

Increasing paid maternity leave[426] in Ireland may help to increase rates of breastfeeding and it is recommended that a review of work place support, policies and facilities is undertaken to encourage mothers to continue to breastfed once they return to work.

UNICEF[6, 391], Food Safety Authority of Ireland[67] and UK's Scientific Advisory Committee on Nutrition[5] are some of the international organisations that recommend that children under one year of age, who are not exclusively breastfed, are provided with whey-based infant formula. Most studies to date which document infant feeding have examined if breastfeeding recommendations are being met, but have not examined the type of infant formula provided to the infant. This PhD found that infants are exposed to a variety of infant formula by the time they are two months old. Just over half (62.4%) of formula-fed infants were being given a whey-based infant formula at two months, and this dropped to 40.4% at six months and 12.8% at 12 months. There was no consistent parental or infant characteristic associated with using a whey-based infant formula throughout the first year of life. Parental perception of infant's hunger was the initial reason for changing infant formula in the first six months of life but by 12 months it was commercial marketing that influenced most parents to change their child's infant formula. Infants that were initially breastfed were most likely to be placed on follow-on infant formula and from the qualitative data it was reported by parent(s) that they followed the advice on the package and believed that they needed to change infant formula to match their child's age. This is a key public health finding – parent(s) were using the marketing information from infant formula companies to decide what formula was suitable for their child. The package (labelling information) of infant formula does not state infant formula guidelines and therefore parent(s) are using the promotional material provided by infant formula companies on deciding on which infant formula to buy. This is a finding that requires additional attention, and may be amenable to a public health education intervention.

Data from The Baby Milk Study, RCT from the MRC Epidemiology Unit based in the University of Cambridge, UK, may be able to help address some of these points. The Baby Milk Study was established to determine the information needs of parent(s) with formula-feeding their child. They randomised parent(s) to either receive new infant feeding information or current advice and collected information on infant feeding, type of infant formula, behaviour (including appetite) and growth[427]. This data has recently become available to researchers, and will allow a more specific insight into maternal attitudes and knowledge around infant formula to further direct how to address the use of marketing by companies as a form of feeding information for parents.

In our analysis of body composition, growth and neurodevelopment analysis, formula fed infants were only included if they never received any breastmilk since birth. This was to remove any cross-over effects that may occur between infants that experienced both exposures[10]. No direct association was found between being breastfed or formula-fed and obesity at two years of age. In the primary analysis for all infants, male or female, formula-feeding increased their WFL z-score change in the first two months of life and rates of ERG were higher compared to breastfeeding infants. ERG was shown to increase WFH z-score at two years for male infants. However, in exploring these findings using interaction models, in the supplementary analysis, the joint moderating effect of sex, how the infants were fed in the first two months of life and WFA z-score change in the first two months of life did statistically influence their WFA z-score at two years of age. This was examined further using interaction models and in comparison to all other multivariate analysis being breastfed did not reduce the WFA z-score change in the first two months of life. Overall, using three different methods to control for both sex and feeding group there is evidence that method of infant in the first few months of life does influence, through the causal pathway, a child’s size at two years of age. Although it should be remembered that only two methods (adjusted and stratified) found a direct link between method of feeding and WFA z-score change in the first two months of life.

For male and female infants, none of the macronutrients in formula feeds assessed were associated with body composition changes from birth to two months or at two months or obesity at 24 months. For male infants the carbohydrate content positively correlated with WFL z-score change in the first two months of life.

No association was found based on feeding group or experience of ERG/ WFL z-score change in the first two months of life on communication, gross and fine motor skills, problem solving or personal social skills at 24 months. None of the maternal or infant characteristics associated with WLH z-score at 24 months or ERG influenced any of the neurodevelopment domains at 24 months. Exclusively formula-fed infants experienced more ERG, had higher WFH z-score at 24 months but all five neurodevelopmental domains were similar to their breastfeeding counterparts. The increased risk of developing later-onset components of the metabolic syndrome was not off-set by neurodevelopmental gains.

This is not a surprising finding. The cohort for this PhD are all healthy, term infants born to primiparas. It was not expected that method of feeding or growth rate would have a negative impact on their neurodevelopment. However, it cannot be stated that no cognitive differences will exist between the two groups. This PhD used the ASQ – third edition which screens, not evaluates, the developmental abilities of children. Neurodevelopment progress is a surrogate marker for later intelligence, executive function and academic success. We cannot rule out more subtle differences between the two groups which may develop in later years, or might be detected using direct measures of developmental progress rather than parental report.

I have also shown that, in our cohort, differences do exist between breastfed and formula-fed infants at birth, prior to the exposure. When looking at the effect of an infant’s milk on their body composition and growth it was noted that breastfed infants had a significantly lower %FM at birth compared to formula-fed infants. In exploring this further, I found that during the antenatal scan, formula-fed infants had a significantly higher abdominal circumference at 20 gestational weeks' compared to breastfed infants. At birth, breastfed infants, although heavier, had lower absolute and relative FM compared to formula-fed infants. Previous studies have investigated for differences in birthweight but our results show that how the fetal weight is gained during pregnancy differs between infants that, once born, will become 'breastfed' or 'formula-fed' infants. This could reflect differences, between breastfeeding and formula-feeding groups, in maternal and environmental characteristics that could impact on the uterine environment.

In this cohort, differences across the groups (other than gender) that were present prior to the infant being breastfed or formula fed, were maternal BMI at 15 gestational weeks' and tertiary education. Maternal overweight/obesity at 15 gestational weeks' significantly increased an infant's FM(kg) across all centiles compared to mothers with a normal BMI and over twice as many formula-fed infants had a mother with an overweight/obsess BMI at 15 gestational weeks' compared to breastfeeding infants. Data on maternal glucose was only available for those that were screened by the maternity hospital and therefore this PhD was unable to examine if the effects of maternal BMI are mediated via glucose levels, as shown by Henriksson et al (2015)[304].

Results from studies identified in the literature search showed that mothers who gained excessive weight during pregnancy as per the IOM guidelines gave birth to infants with a higher %FM[191, 288, 291]. In our cohort excessive weight gain significantly increased an infant's FM(kg) and %FM across all centiles compared to weight gain as per IOM guidelines. Although formula-feeding mothers were more likely to have excessive weight gain (42.6%) compared to breastfeeding mothers (35.8%) this difference was not statistically significant.

Rates of smoking differed across the groups. Infants born to mothers who smoked did have a higher %FM but the mean difference was 0.21%. This does not mean that smoking does not influence neonatal body composition; only two breastfeeding mothers smoked in pregnancy and overall, only a tenth of the total maternal study population reported smoking in pregnancy. The small sample size of smokers could explain the small effect size of smoking in pregnancy on neonatal body composition.

Therefore, it is likely that the difference in birth weight and body composition seen is due to a combination of maternal factors, each having an additive effect, providing a bundle of obesogenic care to the infant. The impact of the contrasting uterine environments could result in differences in the body composition of neonates before they become ‘breastfed’ or ‘formula-fed’ infants. How the uterine environments influences the body composition at birth needs to be taken into account when assessing the effects of infant feeding on growth.

Limitations to this PhD: Selection bias needs to be considered in the interpretation of my results. Although at recruitment, for a prospective cohort, the outcome of interest has not occurred, differences can be noted between those that do and do not consent to participate and who remains in the study, once consented. This PhD is a secondary analysis of the data collected as part of the SCOPE Ireland and Cork BASELINE Birth Cohort Studies. The factors that influence infant feeding, body composition, growth and neurodevelopment are multifactorial. Data that was not collected and would have benefited this PhD include that all mothers (and not just those at risk) were screened for GDM, maternal feeding intentions, and support for same, parents and health care professionals’ knowledge on infant formula feeding guidelines, age of infant when exclusively breastfeeding stopped and when infant formula was changed.

Research outputs from this thesis: This thesis has produced several papers (published and submitted). The first paper from this PhD ‘Early life factors associated with the exclusivity and duration of breast feeding in an Irish birth cohort study[428]' was selected as one of the Editor's Collection – special selection of papers from 2015[429]. Since this work was published other papers have examined the impact of maternity leave and hospital maternity care on breastfeeding practices and rates (and referenced the findings from this PhD[426, 430-435].

I was an invited speaker to the WHO's Guideline Development Group meeting 'Nutrition Actions 2016-2018', which took place between 7th - 11th of November 2016 in Florence, Italy and I contributed to the WHO's 2017 publication 'Protecting, promoting and supporting breastfeeding in facilities providing maternity and newborn services'[436].

The second paper 'Infant formula feeding practices in a prospective population based study[437]' has been forwarded by networks, such as the Trent Perinatal and Central Newborn Networks, to their members. I have also been invited to discuss the findings from this paper to dietetic departments and now sit on the breastfeeding committee for the largest paediatric hospital on the island of Ireland, Our Lady's Children's Hospital, Crumlin.

The remaining papers are submitted for publication and are currently under review. Results from these papers have been presented at both national and international conferences. Through my work examining the body composition of neonates I was invited to contribute to Dr Darren L Dahly's paper 'Associations between maternal lifestyle factors and neonatal body composition in the Screening for Pregnancy Endpoints (Cork) cohort study[438]' which was published by the International Journal of Epidemiology. Following on from this work both Dr Dahly and myself are working on a systematic methodology review of studies which have measured body composition in the first two years of life.

Chapter 9: Conclusion

9.1 Key Findings from PhD

This PhD has shown:

1) Breastfeeding recommendations in Ireland are not being meet. Less than half (n=469) of breastfed infants left the maternity hospital exclusively breastfed and by six months only seven infants were exclusively breastfed as per WHO guidelines. Formula supplementation in the maternity hospital and admission to the NICU, but not reason for admission, had the greatest negative impact on breastfeeding rates. For working mothers, the duration of maternity leave influenced how long they breastfed their child. For infants given formula, the majority, by two months of age, were on non-whey based infant formula. Parental perception of infant hunger and marketing from infant formula companies had the greatest influence on parents changing to non whey-based infant formula.
2) Formula-fed infants experienced greater rates of ERG and ERG increased WFH z-score at 24 months of age. No neurodevelopment differences were seen between breast- and formula-fed infants at 24 months.
3) Differences, in body composition, between breast- and formula-fed infants are present at birth, prior to the exposure. Breastfed infants have a significantly lower %FM compared to formula-fed infants. In this cohort the mothers who formula-fed their infants had higher rates of maternal overweight/obesity at 15 gestational weeks. Infants born to mothers who were overweight/ obese at 15 gestational weeks' had higher %FM at birth, compared to infants born to mothers with a normal BMI at 15 gestational weeks'.

9.2 Recommendations

One of the findings from this PhD suggested that infant feeding may not be a main driver in the variation of infant body composition and growth trajectory.

However, given the limitations of this PhD it is recommended that these results are examined in another study. The methodology of the second study will be similar to this thesis but will also capture:

- At 20 gestational weeks' record the following information:
  - maternal infant feeding intentions (duration of exclusive and any breastfeeding)
  - if the mother believes she is supported in her infant feeding intentions by her mother, partner and close friends
  - what antenatal education the mother has or will receive to support her infant feeding intentions
  - maternal knowledge surrounding infant feeding (both breastfeeding and infant formula) guidelines
- At 28 gestational weeks' all primiparous to be tested for GDM
- At the first neonatal assessment, within 72 hours after birth, also report:
  - maternal infant feeding intentions (duration of exclusive and any breastfeeding)
  - if the mother believes she is supported in her infant feeding intentions by her mother, partner and close friends
  - what antenatal education the mother has received to support her infant feeding intentions
  - maternal knowledge surrounding infant feeding (both breastfeeding and infant formula) guidelines
- At discharge from the maternity hospital extract from the medical charts the time, type and duration of first feed; types of infant feeding (exclusive breastfeeding, breastfeeding with infant formula and exclusive infant feeding) and indication for same during hospital stay
- Mothers to record the dates when exclusive breastfeeding stopped, infant formula change (including reason) and when solid food introduced for the child's first six months of life

There is also a wealth of data to be explored from the Baby Milk Study and it is now open to external researchers to submit requests for data. Although all results will be from a secondary analysis the Baby Milk Study has collected information on infant formula, appetite, behaviour and growth on 622 infants in the first six months of life and 586 in the first year of life[439]. There is the opportunity to use pre-existing data to explore in much greater detail parents' knowledge on the different types of infant formula and reasons why they select, and possible change, infant formula. The data would also present the opportunity to explore if the volume of feeds change if the parent(s) move to a different infant formula. The impact of parental knowledge and practice can be examined against the growth patterns of their infants.

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Appendix 1: Abbreviations

AAP	American Academy of Pediatrics
ADP	Air displacement plethysmography
ARA	Arachidonic acid
ASQ	Ages & Stages Questionnaire
BASELINE	Babies After SCOPE: Evaluating the Longitudinal Impact using Nutritional and Neurological Endpoints
BCAA	Branched chain amino acids
BF	Breastfed/ breastfeeding
BMI	Body mass index
CDI	Child Development Inventory
CI	Confidence interval
cm	Centimetres
CVD	Cardiovascular disease
DHA	Docosahexaenoic acid
DXA	Dual x-ray absorptiometry
EAACI	European Academy of Allergy and Clinical Immunology
ECHO	Ending Childhood Obesity
EDEN	Étude des Déterminants pré et postnatals du développement et de la santé de l’Enfant
ESPGHAN	The European Society for Paediatric Gastroenterology Hepatology and Nutrition
FM	Fat mass
%FMI	Percentage fat mass
FFM	Fat free mass
FSA	Food Standards Agency
g	Grams
GDM	Gestational diabetes mellitus
GTT	Glucose tolerance test
GUI	Growing Up In Ireland
GW	Gestational weeks’
GWG	Gestational weight gain

HbA1c	Glycated hemoglobin
HCP	Healthy Child Programme
HSE	Health Service Executive
IFS	Infant Feeding Survey
IGF-1	Insulin-like growth factor 1
IFPS II	Infant Feeding Practice Study II
INTERGROWTH-21st Project	International Fetal and Newborn Growth Consortium for the 21st Century
IOM	Institute of Medicine
IUGR	Intrauterine growth restriction
Kg	Kilograms
LCPUFA	Long-chain polyunsaturated fatty acids
LISA	Influences of life-style factors on the immune system and the development of allergies in childhood
mg	Microgram
mmHg	Millimeters of mercury
MGRS	Multicentre Growth Reference Study
MRI	Magnetic resonance imaging
mTORC1	Mammalian target of rapamycin complex 1
NCRC	National Children's Research Centre
NCSS	Newborn Cross-Sectional Study
NICU	National Intensive Care Unit
Non-WBF	Non whey-based infant formula
OR	Odds ratio
PEDS	Parents' Evaluation of Developmental Status
PHN	Public Health Nurses
RCTs	Randomised control trials
SCOPE	Screening for Pregnancy Endpoints
SD	Standard deviation
SES	Socioeconomic status
SGA	Small for gestational age
TBK	Total body potassium

TBW	Total body water
UCC	University College Cork
UK	United Kingdom
WBF	Whey-based infant formula
WHO	World Health Organization

Appendix 2: Literature Search Strategy

1. Methods to measure (isotope dilution, MRI, DXA and ADP) and predictors of neonatal body composition

Inclusion criteria: Studies were included if they examined the reliability and validity of isotope dilution, MRI, DXA and ADP with infants at birth and/or two months of age. Papers that measured body composition at birth or two months of age, using any of the selected methods, were also examined to see if they reported on the predictors or reference data of body composition. No limitations were placed on studies based on their design.

How studies were selected: Studies were selected based on the following sequence: (1) Title (2) Abstract and (3) Full paper. All abstracts were reviewed and full text examined if relevant to the topic based on title content.

Additional search strategy: The reference list of eligibility studies were checked to identify any other possible papers

Database searched: Pubmed, CINAHL, Web of Science, Embase, Scopus, TRIP, Google Scholar

Search Terms: (("body composition"[tiab] OR "body composition"[mesh]), ("direct measurement"[tiab] OR "pea pod"[tiab] OR "dual-energy X-ray absorptiometry"[tiab] OR "dxa scan"[tiab] OR "air displacement plethysmography"[tiab] OR "plethysmography"[mesh] OR MRI[tiab] OR "magnetic resonance"[tiab] OR "Absorptiometry"[tiab] OR "computed tomography"[tiab] OR "magnetic resonance"[tiab] OR radiography[mesh]) ("term birth"[Mesh] OR "full-term"[tiab] OR "term infant"[tiab] OR "term birth"[tiab] OR "infant, newborn"[mesh] OR neonate[tiab] OR neonatal[tiab] OR neonates[tiab] OR newborn[tiab] OR newborns[tiab])

Limitations: (1) Language - English; (2) Age – birth to one month; (3) Subject type – human

Month/ Year search was completed: December 2014

2. Predictors of growth (weight, length/height, head circumference) from birth to 2 years of age

Inclusion criteria: Studies were included if they examined antenatal or early feeding predictors of infant body composition at birth and two months of age. No limitations were placed on studies based on their design.

How studies were selected: Studies were selected based on the following sequence: (1) Title (2) Abstract and (3) Full paper. All abstracts were reviewed and full text examined if relevant to the topic based on title content.

Additional search strategy: The reference list of eligibility studies were checked to identify any other possible papers

Database searched: Pubmed, CINAHL, Web of Science, Scopus, TRIP, Google Scholar

Search Terms: (Anthropometry"[majr], "Body Mass Index"[majr] ,"Body composition"[majr], "Body Weights and Measures""[majr], "Child Development/physiology"[majr], "Fetal Development"[majr], "Embryonic and Fetal Development"[majr], "Growth"[majr], "Reference Standards"[majr], "Weight Gain"[majr] "Body Height"[majr], "Body Size"[majr], "birth weight"[majr] , "Body Weight"[majr], "Cephalometry"[majr], "crown-rump length"[majr], "Head/embryology"[majr], "Head/growth and development"[Majr] , "weight-for-age"[tiab], "length-for-age"[tiab], "birth weight-for-gestational age"[tiab], "z-scores"[tiab], "z-score"[tiab], "biparietal diameter"[tiab], "bi-parietal diameter"[tiab], "Kaup index"[tiab], "Quetelet index"[tiab], "birth weight"[tiab], height[tiab], weight[tiab], length[tiab], "head circumference"[tiab], "body size"[tiab], “crown-rump length"[tiab], cephalometry[tiab], Weight Gain[majr], Body Mass Index[majr], Adipose Tissue[mesh, Overweight/epidemiology[mesh], growth trajectories[tiab], childhood growth[tiab], infant growth[tiab], postpartum growth[tiab], antenatal[tiab], postnatal growth[tiab], intrauterine[tiab], in utero[tiab], pregnancy[tiab], pregnancy-related[tiab], maternal factors[tiab], trajectories of body mass index z scores[tiab], Lifecourse analysis[tiab], Longitudinal studies[mesh], Cross-Sectional Studies[mesh], cohort studies[mesh], catch-up growth[tiab], catch-down growth[tiab], gestational weight gain[tiab], Educational Status[majr], Socioeconomic Factors[mesh], socioeconomic[tiab], ethnicity[tiab], ethnic[tiab], smoking[majr], "Electronic Cigarettes"[majr], "tobacco use disorder"[majr], "tobacco products"[majr], "tobacco use"[majr] , “Tobacco Smoke Pollution”[majr] , smoking[tiab], cigarett*[tiab], tobacco[tiab], diet[tiab], 0-2 years[tiab], 24 months[tiab], neonat*[tiab], infant[tiab], Infant[mesh], Generation R study[tiab], ABCD study[tiab], National Longitudinal Survey of Youth (NLSY)[tiab], EDEN cohort study[tiab], NINFEA[tiab], GOCS[tiab], GXXI[tiab], Hong Kong Chinese birth cohort "Children of 1997"[tiab], IDEFICS Study[tiab], ALSPAC study[tiab], Avon Longitudinal Study of Parents and Children[tiab], Early Childhood Longitudinal Study – Birth Cohort[tiab]NOT("Premature Birth"[majr], "Infant, Premature"[majr] OR "prematurity"[ti], "premature"[ti] OR "preterm"[ti] , "adolescent"[mesh] , adolescen*[ti])

Limitations: (1) Language - English; (2) Age – birth 23 months; (3) Subject type - human

Month/ Year search was completed: March 2015

3. Predictors of neurodevelopment at 2 years of age

Inclusion criteria: Studies were included if they examined antenatal or early feeding predictors of neurodevelopment skills at two years of life. No limitations were placed on studies based on their design.

How studies were selected: Studies were selected based on the following sequence: (1) Title (2) Abstract and (3) Full paper. All abstracts were reviewed and full text examined if relevant to the topic based on title content.

Additional search strategy: The reference list of eligibility studies were checked to identify any other possible papers

Database searched: Pubmed, CINAHL, Web of Science, Embase, Scopus, TRIP, Google Scholar

Search Terms: (Human Development/complications"[Majr] OR "Nervous System Physiological Phenomena/abnormalities"[Majr] OR "Nervous System Physiological Phenomena/complications"[Majr] OR "Nervous System Physiological Phenomena/deficiency"[Majr] OR "Nervous System Physiological Phenomena/drug effects"[Majr] OR "Nervous System Physiological Phenomena/growth and development"[Majr] OR "Nervous System Diseases"[Majr] OR "Psychological Tests"[Majr] OR "Mental Disorders Diagnosed in Childhood"[Majr] OR "Neurologic Examination"[Majr] OR "Cognition Disorders"[Majr] OR "Adolescent Development"[Majr] OR "Language Development"[Majr] OR "Learning"[Majr] OR "Adaptation, Psychological"[Majr] OR "Early Intervention (Education)"[Majr] OR "Dominance, Cerebral"[Majr] OR "Brain/abnormalities"[Majr] OR "Brain/adverse effects"[Majr] OR "Brain/growth and development"[Majr] OR "Diagnostic Techniques, Neurological"[Majr] OR "Intelligence"[Majr] OR “Intelligence tests”[mesh] OR "Disability Evaluation"[Majr] OR " Psychology, Child "[Majr] OR " Psychology, Adolescent "[Majr] OR "Education, Special"[Majr] OR “child development”[tiab] OR “adolescent development"[tiab] OR "infant development"[tiab] OR "brain development"[tiab] OR "developmental outcome"[tiab] OR "developmental delay"[tiab] OR "developmental disability"[tiab] OR "developmental score"[tiab] OR "developmental status"[tiab] OR "development scores"[tiab] OR "developmental deficits"[tiab] OR neurodevelopment[tiab] OR neurodevelopmental[tiab] OR neurobehavioral[tiab] OR neuropsychology[tiab] OR neuropsychological[tiab] OR "mental disabilities"[tiab] OR "mental disability"[tiab] OR "child behaviour"[tiab] OR "child behavior"[tiab] OR "child psychology"[tiab] OR "cognitive performance"[tiab] OR "learning disabilities"[tiab] OR "learning difficulties"[tiab])

Limitations: (1) Language - English; (2) Age – birth to 23 months; (3) Subject type - human

Month/ Year search was completed: May 2015

4. Milk diet of infants in the first 12 months of life

Inclusion criteria: National infant feeding surveys examining the milk diet of infants in the first 12 months of life undertaken in developed countries. No limitations were placed on studies based on their design.

How studies were selected: Studies were selected based on the following sequence: (1) Title (2) Abstract and (3) Full paper. All abstracts were reviewed and full text examined if relevant to the topic based on title content.

Additional search strategy: The reference list of eligibility studies were checked to identify any other possible papers

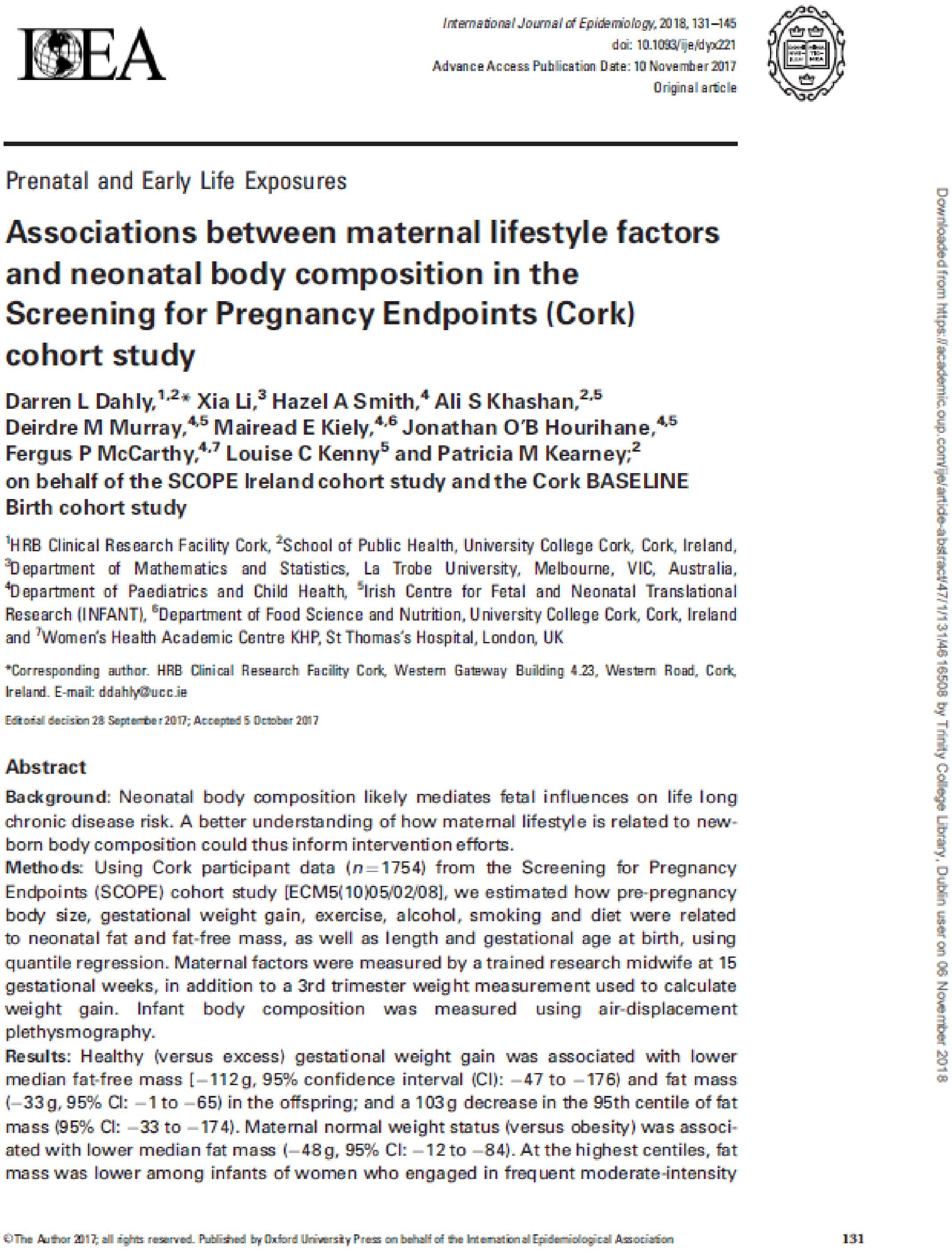
Database searched: CINAHL Plus w Full Text, Web of Science, ScienceDirect

Search Terms: ("infant formula"[mesh] OR "infant formulas"[mesh] OR "artificial feeding" [mesh] OR "bottle feeding" [mesh] OR "bottle feedings" [mesh] AND "breastfeeding" [mesh])

Limitations: (1) Language - English; (2) Age – birth to 23 months; (3) Subject type - human

Month/ Year search was completed: December 2012

Appendix 3: ‘Associations between maternal lifestyle factors and neonatal body composition in the Screening for Pregnancy Endpoints (Cork) cohort study’



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Prenatal and Early Life Exposures

Associations between maternal lifestyle factors and neonatal body composition in the Screening for Pregnancy Endpoints (Cork) cohort study

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Abstract

Background: Neonatal body composition likely mediates fetal influences on life long chronic disease risk. A better understanding of how maternal lifestyle is related to newborn body composition could thus inform intervention efforts.

Methods: Using Cork participant data (*n* = 1754) from the Screening for Pregnancy Endpoints (SCOPE) cohort study [ECM5(10)05/02/08], we estimated how pre-pregnancy body size, gestational weight gain, exercise, alcohol, smoking and diet were related to neonatal fat and fat-free mass, as well as length and gestational age at birth, using quantile regression. Maternal factors were measured by a trained research midwife at 15 gestational weeks, in addition to a 3rd trimester weight measurement used to calculate weight gain. Infant body composition was measured using air-displacement plethysmography.

Results: Healthy (versus excess) gestational weight gain was associated with lower median fat-free mass [−112 g, 95% confidence interval (CI): −47 to −176) and fat mass (−33 g, 95% CI: −1 to −65) in the offspring; and a 103 g decrease in the 95th centile of fat mass (95% CI: −33 to −174). Maternal normal weight status (versus obesity) was associated with lower median fat mass (−48 g, 95% CI: −12 to −84). At the highest centiles, fat mass was lower among infants of women who engaged in frequent moderate-intensity exercise early in the pregnancy (−92 g at the 95th centile, 95% CI: −168 to −16). Lastly, women who never smoked tended to have longer babies with more fat mass and fat-free mass. No other lifestyle factors were strongly related to infant body composition.

Conclusions: These results suggest that supporting healthy maternal lifestyles could reduce the risk of excess fat accumulation in the offspring, without adversely affecting fat-free mass development, length or gestational age.

Key words: Developmental origins, body composition, lifestyle, birth cohort, quantile regression

Key Messages

- Maternal body size and gestational weight gain were most strongly associated with newborn body composition.
- Associations between maternal lifestyle and newborn body composition were often stronger in the tails of the outcome's distribution.
- Quantile regression provided useful insights that would not have been apparent with linear or logistic regression.

Introduction

The environment experienced by a fetus during development influences its risk for cardiovascular disease, diabetes and obesity across the life course.[1–4] Healthy maternal lifestyles before and during pregnancy may affect fetal development and thus influence these outcomes,[5,6] but our understanding is limited since many birth cohort studies capable of linking maternal factors to cardiometabolic risks in the offspring are not yet into middle age.[7,8] However, newborn body composition is a useful reflection of the fetal environment in this context,[9,10] and likely mediates developmental effects on long-term cardiometabolic outcomes.[7,11,12] Thus a better understanding of how maternal lifestyle is related to newborn body composition could provide important insights into the developmental origins of adult health and disease.

Some of the challenges in measuring fat and fat-free mass in infants have recently lessened,[13] facilitating relatively large population-based studies of infant body composition.[14–18] Another challenge is how to best model the impact of one or more predictors on a single continuous anthropometric measure where we expect distinct aetiological mechanisms at opposite ends of the measure's distribution (e.g. the causes of low birthweight versus those of macrosomia). This is typically approximated by categorizing the otherwise continuous variable and investigating it using binary or multinomial logistic regression. This approach is useful to the degree that the categorisation is clinically relevant, but it ignores the continuous nature of the variable and inevitably discards useful information about the rank order of individuals in the sample.

To avoid this, we used quantile regression[19] to estimate associations between maternal lifestyle factors and offspring neonatal fat mass and fat-free mass in one of the largest relevant studies to date. Quantile regression is analogous to multiple linear regression, but instead of modelling the mean of the dependent variable conditional on the predictor(s), it can be used to similarly model any centile. Importantly, quantile regression allows you to estimate how the tails of a variable's distribution (such as the 5th or 95th centile) vary across levels of a predictor, which can occur even when there is no obvious shift in the mean or median. This allowed us to test the hypothesis that infants born to mothers exhibiting healthier lifestyle factors would be less likely to have very high or low levels of fat and fat-free mass.

Methods

Ethical standards

The authors assert that all procedures contributing to this work comply with the International Ethical Guidelines for Epidemiological Studies (CIOMS/WHO) and with the Helsinki Declaration of 1975, as revised in 2008, and has been approved by the Research Ethics Committee of the Cork Teaching Hospitals provided for the SCOPE-Ireland study [ref: ECM5(10)05/02/08].

Study and sample

Data are from the Cork site of the Screening for Pregnancy Endpoints (SCOPE) pregnancy cohort study (ACTRN12607000551493), and its follow-up birth cohort study, Babies after

SCOPE: Evaluating the Longitudinal Impact on Neurological and Nutritional Endpoints (BASELINE; ClinicalTrials.gov NCT 01498965; www.birthcohorts.net). The primary aim of SCOPE was to identify clinical factors and biomarkers that were predictive of pre-eclampsia, small for gestational age babies and spontaneous preterm birth. Based on this aim, the study included healthy, nulliparous women with singleton pregnancies, and the exclusion criteria were: known major fetal anomalies; pre-pregnancy essential hypertension; moderate to severe hypertension at booking; pre-existing diabetes; renal disease; systemic lupus erythematosus; antiphospholipid syndrome; HIV positivity; major uterine anomaly; cervical suture; knife cone biopsy; ruptured membranes at recruitment; three or more miscarriages; three or more terminations; long-term steroid use; and treatment with low-dose aspirin, calcium ($>$1000 g/24 h) or Vitamin E ($\geq$400 iu), low-molecular-weight heparin, fish oil or antioxidants. All women who participated in the SCOPE study were informed about the birth cohort, and if consent was obtained infants were registered to the Cork BASELINE birth cohort.

Participants were recruited from Cork University Maternity Hospital between February 2008 and February 2011, at 15 ± 1 weeks of gestation. During this period, 2579 nulliparous women were invited to participate and 1774 (69%) consented to do so. Study participants were interviewed and examined by a trained research midwife at recruitment and again at 20 ± 1 weeks of gestation. Demographic, socioeconomic and medical data were collected, as well as information on current diet, physical activity and other lifestyle factors.

Of these 1774 women, 1537 went on to have an infant enrolled into the BASELINE birth cohort (87%). Infant measures were taken by a trained research midwife within 72 h of birth. All data were managed using an internet accessible database with a clear audit trail and automated quality assurance procedures (MedSciNet AB, Stockholm, Sweden). Additional details on the study's methods have been previously reported.[20,21]

Measurements and variable definitions

Infant measures

Infant measures used in this analysis were body composition, length, weight, gestational age at delivery and sex. Per study protocol, the aim was to measure body composition within 48 h. First, newborn body density was calculated using weight (measured with an electronic scale to the nearest gram), divided by newborn body volume (estimated by air-displacement plethysmography with a PEA POD® Infant Body Composition System, COSMED USA, Concord, CA), giving body density. Based on a two-compartment model of body composition (fat and fat-free mass) and body density values from Fomon,[22] percent body fat was calculated, and was in turn used to estimate fat and fat-free mass in grams, which were the primary outcomes of interest.

Gestational age was based on expected date of delivery, which was estimated from respondent recall of last menstrual period (LMP). If the respondent was uncertain about their LMP, or it differed substantially from a 16- or 20-week scan (by $\geq$7 or $\geq$10 days, respectively), the earliest available scan date was used. Length was measured with a neonatometer to the nearest millimetre.

Maternal lifestyle factors

The maternal lifestyle factors we considered were those that most closely aligned with current pregnancy recommendations regarding nutrition and lifestyle in Ireland.[23] Pre-pregnancy weight (kg) was estimated as weight measured at recruitment, less 1.25 kg [the assumed average amount of weight gain in the first 15 weeks of pregnancy based on the 2009 Institute of Medicine (IOM) guidelines].[24] Pre-pregnancy body mass index (BMI) was calculated from pre-pregnancy weight (kg) divided by measured height squared (m^2). Pre-pregnancy weight status was subsequently categorised based on World Health Organization guidelines as underweight (BMI $<$ 18.5 kg/m^2), normal weight (BMI 18.5 to 25 kg/m^2), overweight (BMI $\geq$ 25 to 30 kg/m^2) and obese (BMI $\geq$ 30 kg/m^2).

In addition to the weight measurement at recruitment, weight was also typically measured multiple times across the pregnancy during routine care. To account for these differences, the weight gain rate (kg/week) was calculated as the difference between the last of these measures (whenever it occurred, usually late in the third trimester) and the initial weight measured at recruitment, divided by the number of weeks between those measures. Excessive gestational weight gain (GWG) was defined according to Institute of Medicine (IOM) guidelines as a weight gain rate exceeding 0.5 kg/week (wk) in underweight and normal weight women, 0.33 kg/wk in overweight women, and 0.27 kg/wk in obese women; and inadequate GWG was defined as a weight gain rate below 0.35 kg/wk in underweight and normal weight women, 0.23 kg/wk in overweight women, and 0.17 kg/wk in obese women.[24,25]

Women were asked at recruitment (15 ± 1 weeks of gestation) how many times each week they engaged in exercise that that did not result in heavier breathing, which was the study's definition of moderate-intensity exercise. Their responses were categorized as Never, Some (1 to 3 times a week) and Often (4 or more times a week). Respondents also reported daily hours of television viewing in the past month, a commonly used marker of sedentary activity[26] particularly in women,[27] which was categorised as $<$2 h, 2 to 4 h and 5 or more h. Based on participant-reported consumption at 15 ± 1 and/or 20 ± 1 weeks of gestation, alcohol use was categorised as Never, Quit before Pregnancy, Quit during Pregnancy and Still Drinking; and smoking was categorised as Never Smoked, Quit during Pregnancy and Still Smoking. Women were asked about pre-pregnancy folic-acid supplementation, and their responses were dichotomized as those meeting the recommended 400µg versus those who did not (Yes versus No).

The questionnaire administered at recruitment asked women to report the frequency with which they consumed several food items in the first 15 weeks of pregnancy. Their responses were used to determine whether they were meeting the recommended five servings of fruit and veg per day (Yes versus No) and at least 1 serving of oily fish per week (Yes versus No).

Covariates

Available covariates likely to influence both maternal lifestyle and birth outcomes were selected based on the expert opinion of the study authors, who were also careful to not select covariates that were probable consequences of maternal lifestyle. All selected covariates were assessed at 15 ± 1 weeks' gestation, and included: maternal age (years); the mother's reported weight at birth (g); her gravidity (1 versus > 1); her ethnicity (White versus Non-white); whether she had a partner or not (Yes versus No); whether she had any third level education (Yes versus No); whether she used Public versus

Table 1. Characteristics of 1754 sample mother-infant pairs enrolled in SCOPE-Ireland, 2008 to 2011

Variable	Missing Values	Proportion (n)	Mean (SD)	Median[IQR]	Range
Infant characteristics					
Sex	0				
Male		0.51(892)			
Female		0.49(862)			
Birth weight (g)	0		3462 (507.5)	3460 [3150 to 3778]	1200 to 5130
Fat mass (g)	512		378 (172.9)	351 [253 to 481]	36 to 1099
Fat free mass (g)	513		2955 (346.8)	2965 [2730 to 3182]	1848 to 3960
Percent fat mass	514		11.1 (4.1)	10.9 [8.2 to 13.8]	1.3 to 30.1
Length (cm)	59		50.2 (2.4)	50.2 [49 to 51.8]	37.5 to 57
Gestational age (wks)	0		40 (1.5)	40.3 [39.3 to 41]	32 to 42.6
Maternal characteristics					
Age	0		29.9 (4.5)	30 [28 to 33]	17 to 45
Height	0		164.6 (5.9)	165 [161 to 168]	147 to 185
Birth weight	60		3360.8 (532.9)	3374 [3062 to 3657]	624 to 6000
Gravidity	0				
1		0.85(1483)			
2+		0.15 (271)			
Ethnicity	0				
White		0.98(1712)			
Nonwhite		0.02(42)			
Has partner	0				
Single		0.11(186)			
Partner		0.89(1568)			
3rd level education	0				
No		0.11(195)			
Yes		0.89(1559)			
SEI†	0		42.7 (16)	45 [29 to 51]	18 to 89
Maternity care	0				
Public		0.75(1318)			
Private		0.25(436)			
Depressed	0				
Unlikely		0.41(711)			
At risk		0.35(622)			
Likely		0.24(421)			
Stress score ††	0		13.7 (6.6)	13 [9 to 18]	0 to 35

IQR, interquartile range; SD, standard deviation; SEI, Socioeconomic index.

†Based on the New Zealand socioeconomic index, with higher values reflecting greater social status.

††Out of a maximum score of 40, with higher scores reflecting higher levels of stress.

Private maternity care; her socioeconomic index (SEI), based on the New Zealand SEI[28] (with higher values reflecting higher social status); her risk of depression based on the Edinburgh Postnatal Depression Score[29,30] (Unlikely to experience depression versus At risk of depression in the next year versus Likely depressed); and her score on the Perceived Stress Scale[31] (with higher scores reflecting greater stress).

Table 2. Maternal lifestyle factors in 1754 sample mothers enrolled in SCOPE-Ireland, 2008 to 2011

Variable	Missing values	Proportion (n)
Prepregnancy body size	0	
Obese (BMI ≥ 30 kg/m^2)		0.11 (190)
Overweight (BMI 25 to 30 kg/m^2)		0.24 (419)
Normal weight (BMI < 25 kg/m^2)		0.65 (1145)
IOM defined gestational weight gain level	525	
Excessive		0.79 (977)
Healthy		0.16 (199)
Inadequate		0.04 (53)
Frequency of moderate intensity exercise	0	
None		0.25 (441)
Some		0.55 (965)
Often		0.2 (348)
Amount of daily TV viewing	0	
≥5 h		0.09 (158)
2–4 h		0.55 (958)
<2 h		0.36 (638)
Alcohol use	0	
Still drinks		0.16 (288)
Quit during pregnancy		0.65 (1133)
Quit prepregnancy		0.09 (166)
Never drank		0.1 (167)
Any smoking	0	
Still smokes		0.1 (174)
Quit during pregnancy		0.18 (307)
Never smoked†		0.73 (1273)
Takes folate	0	
No		0.32 (560)
Yes		0.68 (1194)
Eats ≥ 5 servings fruit and veg per day	0	
No		0.86 (1508)
Yes		0.14 (246)
Eats ≥ 1 serving oily fish per week	0	
No		0.69 (1205)
Yes		0.31 (549)

BMI, body mass index; IOM, Institute of Medicine.

†Six women who reported quitting prior to pregnancy were classified as Never Smoked.

Statistical methods

Categorical variables were described by the count and proportion in each category. Continuous variables were described by: their mean and standard deviation; their median and the interquartile range; and their full range.[32]

Relationships between maternal lifestyle factors and infant outcomes were estimated using quantile regression.[19] We first estimated the crude association of each lifestyle factor with each outcome, at every centile from the 2nd to 98th. We then estimated a similar set of fully adjusted models with each outcome regressed on all the lifestyle factors and covariates. The latter were included to account for possible confounding. Based on previous research,[33] we also tested for an interaction between maternal pre-pregnancy weight status and IOM-classified healthy GWG. All continuous covariates were centred at their means. Quantile regression coefficients were estimated using a modified version of the Barrodale and Roberts algorithm,[34] and standard errors (SE) for coefficients were calculated using the kernel-based method suggested by Powell.[35]

Missing data were handled using multiple imputation. Thirty imputed datasets were created, after a burn-in of 30 replications, using predicted mean matching.[36] The imputation model included all variables included in this analysis, and allowed for non-linear relationships using restricted cubic splines with five knots. We took the' transform then impute' approach recommended by von Hippel[37] to impute variables derived from other variables with missing values. All models were estimated using each imputed dataset, and parameter estimates were combined using Rubin's rules.[38] Differences in proportions or means across sub-groups with and without missing data were tested using, respectively, Pearson's chi-square test or Welsh's t-test with unequal variances. Distributions of imputed values were examined visually.

All analyses were conducted using the R Project for Statistical Computing[39] (version 3.1.2). Quantile regression models were estimated using the quantreg package[40] (version 5.11). Multiple imputation was implemented with the Hmisc package[41] (version 3.14–6). All plots were produced using the ggplot2 package[42] (version 1.0.0).

Results

Of the 1774 recruited mothers, three experienced a fetal loss before 20 weeks' gestation; five pregnancies resulted in stillbirths; and 12 infants were born before 32 weeks' completed gestation; these were excluded from the final analytical sample of 1754 infants.

Variable distributions are described in Tables 1 and 2. The infants' anthropometrics and gestational ages were

Table 3. Quantile regression results from the fully adjusted model for fat-free mass (g), n = 1754

	Centile					
	5th		50th		95th	
Variable	β (g)	95% CI	β (g)	95% CI	β (g)	95% CI
Intercept	2444.4	(2153.9 to 2734.9)	3011.4	(2864.3 to 3158.5)	3587.9	(3387.9 to 3787.9)
Healthy GWG	−38.5	(−173.1 to 96.1)	−111.7	(−176.2 to −47.2)	−109.8	(−186.7 to −32.9)
Inadequate GWG	34.7	(−181.2 to 250.6)	−57	(−165.6 to 51.6)	−83.2	(−240.3 to 73.9)
Excessive GWG	ref	–	ref	–	ref	–
Normal weight (BMI < 25 kg/m2)	−166	(−295.8 to −36.2)	−35	(−100.2 to 30.2)	−121.6	(−249.8 to 6.6)
Overweight (BMI 25 to 30 kg/m2)	−123.3	(−271.5 to 24.9)	−8.4	(−81.1 to 64.3)	−90.6	(−223.5 to 42.3)
Obese (BMI ≥ 30 kg/m2)	ref	–	ref	–	ref	–
Takes folate (≥400 mg) (vs. not)	−1.7	(−110.5 to 107.1)	34.4	(−20.6 to 89.4)	−6	(−76.3 to 64.3)
Some moderate-intensity exercise	75.7	(−58.6 to 210)	38.1	(−15.1 to 91.3)	15.3	(−59.3 to 89.9)
Frequent moderate-intensity exercise	16.1	(−127.8 to 160)	−5.8	(−74.5 to 62.9)	−12.5	(−104 to 79)
No moderate-intensity exercise	ref	–	ref	–	ref	–
2 to 4 hours of television	21.2	(−147 to 189.4)	−24.2	(−103.8 to 55.4)	−75.9	(−176.9 to 25.1)
< 2 hours of television	12.5	(−161 to 186)	−28.2	(−114.4 to 58)	−65.6	(−175.7 to 44.5)
4+ hours of television	ref	–	ref	–	ref	–
Quit drinking during pregnancy	29.7	(−108.7 to 168.1)	4.1	(−56.5 to 64.7)	5.7	(−88.8 to 100.2)
Quit drinking prepregnancy	26.2	(−169 to 221.4)	−44.1	(−130.2 to 42)	−4.6	(−152.5 to 143.3)
Never drank	38.3	(−145 to 221.6)	36.2	(−63.8 to 136.2)	−51.7	(−156.8 to 53.4)
Still drinks	ref	–	ref	–	ref	–
Quit smoking during pregnancy	165.5	(−14.8 to 345.8)	47.7	(−45.6 to 141)	60.1	(−57.2 to 177.4)
Never smoked	130.2	(−39.6 to 300)	81	(−5.3 to 167.3)	78.5	(−22.5 to 179.5)
Still smokes	ref	–	ref	–	ref	–
Eats 5 fruit/veg a day (vs. not)	7	(−117.7 to 131.7)	10.8	(−55.2 to 76.8)	54.6	(−30.6 to 139.8)
Eats ≥ 1 serving of oily fish weekly (vs. not)	−52.6	(−164.7 to 59.5)	18.4	(−28.8 to 65.6)	69.8	(−2.7 to 142.3)

BMI, body mass index; CI, confidence interval; GWG, gestational weight gain.

Models further adjusted for infant sex, maternal age, maternal height, gravidity, ethnicity, whether the mother has a partner, maternal education, socioeconomic index, private/public maternity care, risk of depression, and stress score.

consistent with established norms.[16] Two-thirds of women had a healthy pre-pregnancy weight (BMI < 25 kg/m^2), but only 16% experienced a healthy level of GWG. Whereas 16% of women said they were still consuming alcohol at recruitment, 80% of these women reported drinking one or fewer units per week.

Based on the results from the fully adjusted quantile regressions (Tables 3–6), the conditional median for fat-free mass was 112g less (95% CI −176 to −47) in infants born to women who experienced healthy GWG, compared with those who experienced excessive GWG. This reduction was less extreme at the lower centiles of fat-free mass (Figure 1a and Table 3). Healthy GWG was also associated with a 33 g reduction (95% CI −65 to −1) in median fat mass, and a 103 g reduction (95% CI − 174 to −33) at the 95th centile of fat mass (Figure 1b and Table 4). Birth lengths were roughly 0.6 cm less at all centiles in infants born to women who experienced healthy GWG, though 95% CIs at several centiles included the null hypothesis of no difference (Figure 1c).

Pre-pregnancy normal weight status, compared with women classified as obese, was associated with a 48 g reduction (95% CI −84 to −12) in median fat mass, though 95% CIs at several centiles included the null hypothesis of no difference (Figure 2b and Table 4). Pre-pregnancy weight status otherwise unrelated to outcomes. Frequent bouts of moderate-intensity exercise were associated with a reduction in the upper tail of the fat mass distribution (Figure 3a). For example, the 95th centile of fat mass in infants born to women who exercised frequently was 92 g less (95% CI −168 to −16) than in infants born to women who reported never exercising. The upper centiles of fat mass were also reduced in infants born to women who reported never drinking. For example, never drinking was associated with a 122 g reduction (95% CI −204 to −40) in the 95th centile of fat mass (Figure 4 and Table 4). Babies born to women who never smoked had greater fat-free mass and length, at all centiles (Figure 5).

Table 4. Quantile regression results from the fully adjusted model for fat mass (g), n = 1754

	Centile					
	5th		50th		95th	
Variable	β (g)	95% CI	β (g)	95% CI	β (g)	95% CI
Intercept	121.7	(33.7 to 209.7)	371.5	(298.2 to 444.8)	845	(670.3 to 1019.7)
Healthy GWG	−4.5	(−41.2 to 32.2)	−33.4	(−65.5 to −1.3)	−103.2	(−173.8 to −32.6)
Inadequate GWG	27	(−33.7 to 87.7)	−17.1	(−66.6 to 32.4)	−30.5	(−134.4 to 73.4)
Excessive GWG	ref	–	ref	–	ref	–
Normal weight (BMI < 25 kg/m2)	−42.2	(−86 to 1.6)	−47.7	(−83.7 to −11.7)	−69.1	(−149.9 to 11.7)
Overweight (BMI 25 to 30 kg/m2)	−36.1	(−87.4 to 15.2)	−25.6	(−64.5 to 13.3)	−39.9	(−128.5 to 48.7)
Obese (BMI ≥ 30 kg/m2)	ref	–	ref	–	ref	–
Takes folate (≥400 mg) (vs. not)	−8.4	(−38.8 to 22)	8.6	(−18.1 to 35.3)	−16.6	(−74.8 to 41.6)
Some moderate-intensity exercise	9.8	(−23.1 to 42.7)	4.8	(−21.7 to 31.3)	−36.7	(−111.3 to 37.9)
Frequent moderate-intensity exercise	−4.1	(−46.4 to 38.2)	−0.6	(−33.2 to 32)	−91.9	(−168 to −15.8)
No moderate-intensity exercise	ref	–	ref	–	ref	–
2 to 4 hours of television	−4.3	(−48.3 to 39.7)	12.6	(−27.9 to 53.1)	51.5	(−26.7 to 129.7)
< 2 hours of television	−25	(−67.8 to 17.8)	−6.8	(−50.9 to 37.3)	57.6	(−31.4 to 146.6)
4+ hours of television	ref	–	ref	–	ref	–
Quit drinking during pregnancy	2.2	(−38.3 to 42.7)	−26.8	(−58.6 to 5)	−32.6	(−100 to 34.8)
Quit drinking prepregnancy	−3.3	(−69.1 to 62.5)	−22.4	(−67.4 to 22.6)	−88.9	(−191.3 to 13.5)
Never drank	18.2	(−40.1 to 76.5)	−14.3	(−60.1 to 31.5)	−122.3	(−204.1 to −40.5)
Still drinks	ref	–	ref	–	ref	–
Quit smoking during pregnancy	17.1	(−33.6 to 67.8)	1.9	(−43 to 46.8)	−17.1	(−116.3 to 82.1)
Never smoked	28.8	(−17 to 74.6)	29.1	(−11.7 to 69.9)	29.6	(−54.8 to 114)
Still smokes	ref	–	ref	–	ref	–
Eats 5 fruit/veg a day (vs. not)	5.4	(−34.1 to 44.9)	6.9	(−26.1 to 39.9)	31	(−38.4 to 100.4)
Eats ≥ 1 serving of oily fish weekly (vs. not)	−1.7	(−30.6 to 27.2)	−4	(−27.3 to 19.3)	−21.7	(−69.5 to 26.1)

BMI, body mass index; CI, confidence interval; GWG, gestational weight gain.

Models further adjusted for infant sex, maternal age, maternal height, gravidity, ethnicity, whether the mother has a partner, maternal education, socioeconomic index, private/public maternity care, risk of depression, and stress score.

No other lifestyle factors were strongly related to infant outcomes in the fully adjusted quantile regression models (see Supplementary material, available at *IJE* online). Inclusion of a product interaction term for pre-pregnancy weight status and healthy GWG did not appreciably improve fit in any of the quantile regression models we estimated (likelihood ratio test $P > 0.10$ in all cases). All reported models thus excluded this term. A description of missing data is provided in the Supplementary material, available at *IJE* online.

Discussion

Using data from a large cohort of pregnant women, we used quantile regression to link maternal lifestyle factors to the distributions of fat and fat-free mass in their newborn offspring. Consistent with previous research (described below), maternal obesity and excess GWG were most strongly associated with newborn body composition. Both are established risk factors for macrosomia,[25,43] which supports the idea that the associated long-term cardiometabolic risks result from an excess of substrate available to the fetus, particularly glucose, and subsequent fetal hyperinsulinaemia.[44–46] Whereas newborn body composition is likely a more sensitive reflection of these fetal influences than total mass,[9,10] relatively few studies have looked at the independent effects of GWG and pre-pregnancy body size on infant fat and fat-free mass.

We found that maternal obesity (versus normal weight) was associated with an increase in newborn fat mass, though 95% CIs did not exclude the null hypothesis of zero difference at some centiles. This result is broadly consistent with Hull *et al.*[47] ($n = 306$), Sewell *et al.*[9] ($n = 221$), Carlsen *et al.*[48] ($n = 311$), Au *et al*.49 ($n = 599$), Starling *et al.*[50] ($n = 826$) and Friss *et al.*[51] ($n = 207$), who found that maternal overweight and/or obesity were associated with increased fat mass and/or percentage fat mass.

We also found that IOM-defined excessive GWG was associated with increases in both fat and fat-free mass.

Table 5. Quantile regression results from the fully adjusted model for birth length (cm), $n = 1754$

	Centile					
	5th		50th		95th	
Variable	β (cm)	95 % CI	β (cm)	95 % CI	β (cm)	95% CI
Intercept	(42.8 to 47)	50.5	(49.5 to 51.5)	53.7	(52.4 to 55)	
Healthy GWG	−0.5	(−1.5 to 0.5)	−0.4	(−0.8 to 0)	−0.8	(−1.3 to −0.3)
Inadequate GWG	0.1	(−2.8 to 3)	−0.3	(−1 to 0.4)	−0.1	(−1.1 to 0.9)
Excessive GWG	ref	–	ref	–	ref	–
Normal weight (BMI < 25 kg/m2)	−1.2	(−1.9 to −0.5)	−0.4	(−0.9 to 0.1)	−0.5	(−1.1 to 0.1)
Overweight (BMI 25 to 30 kg/m2)	−0.8	(−1.7 to 0.1)	−0.3	(−0.8 to 0.2)	−0.4	(−1 to 0.2)
Obese (BMI ≥ 30 kg/m2)	ref	–	ref	–	ref	–
Takes folate (≥400 mg) (vs. not)	0.4	(−0.5 to 1.3)	0	(−0.3 to 0.3)	0.2	(−0.2 to 0.6)
Some moderate-intensity exercise	0.7	(−0.1 to 1.5)	0.1	(−0.2 to 0.4)	0	(−0.4 to 0.4)
Frequent moderate-intensity exercise	−0.2	(−1.2 to 0.8)	−0.3	(−0.7 to 0.1)	−0.2	(−0.8 to 0.4)
No moderate-intensity exercise	ref	–	ref	–	ref	–
2 to 4 hours of television	2.2	(0.3 to 4.1)	0	(−0.6 to 0.6)	−0.6	(−1.3 to 0.1)
< 2 hours of television	1.6	(−0.3 to 3.5)	0	(−0.6 to 0.6)	−0.5	(−1.2 to 0.2)
4+ hours of television	ref	–	ref	–	ref	–
Quit drinking during pregnancy	0	(−0.9 to 0.9)	0.1	(−0.3 to 0.5)	0.2	(−0.2 to 0.6)
Quit drinking prepregnancy	−0.2	(−1.3 to 0.9)	−0.3	(−0.8 to 0.2)	−0.3	(−1.1 to 0.5)
Never drank	−0.9	(−2.9 to 1.1)	0	(−0.5 to 0.5)	−0.3	(−0.9 to 0.3)
Still drinks	ref	–	ref	–	ref	–
Quit smoking during pregnancy	1.1	(0.2 to 2)	0.5	(0 to 1)	0.5	(−0.2 to 1.2)
Never smoked	0.6	(−0.3 to 1.5)	0.6	(0.1 to 1.1)	0.7	(0 to 1.4)
Still smokes	ref	–	ref	–	ref	–
Eats 5 fruit/veg a day (vs. not)	0.2	(−0.6 to 1)	0.2	(−0.2 to 0.6)	0.3	(−0.2 to 0.8)
Eats ≥ 1 serving of oily fish weekly (vs. not)	−1.1	(−1.8 to −0.4)	0.1	(−0.2 to 0.4)	0	(−0.4 to 0.4)

BMI, body mass index; CI, confidence interval; GWG, gestational weight gain.

Models further adjusted for infant sex, maternal age, maternal height, gravidity, ethnicity, whether the mother has a partner, maternal education, socioeconomic index, private/public maternity care, risk of depression, and stress score.

This finding was consistent with Au *et al.*[49] who observed that each kg of weight gained during pregnancy was associated with increased percentage fat mass and birthweight, and Carlsen *et al.*[48] and Starling *et al.*[50] who found a similar association with fat and fat-free mass. Crozier *et al.*[52] ($n = 564$) also found that IOM-healthy GWG was associated with reduced fat mass at birth, though they did not control for maternal pre-pregnancy body size. Friis *et al.*[51] was the only relevant study not to observe an association between GWG and infant fat mass.

Sewell *et al.*[9] and Hull *et al.*[47] also found that the apparent association between GWG and infant body composition was modified by maternal weight status, though the nature of this interaction differed between studies. The former observed that weight gain during pregnancy was associated with fat-free mass in the infants born to normal weight women, and with percentage fat mass in infants born to overweight and obese women; the latter found that the increase in percentage fat mass associated with excessive GWG was pronounced in overweight mothers. Our analysis, as well as that by Starling *et al.*,[50] found no evidence of such interactions.

Relative to the previous research just described, the unique contribution of our analysis comes from our use of quantile regression and its ability to estimate the effect of predictors on the tails of an outcome's distribution. This approach complements a previous paper using this sample, which used linear regression to investigate lifestyle predictors of neonatal percentage body fat.[53] For example, we found that excessive GWG was clearly associated with higher newborn fat-free mass, and some evidence that it was associated with a small increase in length. Importantly, these associations were consistent across the respective centiles of fat-free mass and length. This is expected, since GWG reflects changes in multiple tissues of the mother, placenta and developing fetus.[24] This is of course true for fat mass as well, except that the association between GWG and fat mass was more pronounced at the upper end of the fat mass distribution. Further, the observed change in fat mass at the upper end of its

Table 6. Quantile regression results from the fully adjusted model for gestational age (weeks), n = 1754

	Centile					
	5th		50th		95th	
Variable	β (weeks)	95% CI	β (weeks)	95% CI	β (weeks)	95% CI
Intercept	36	(34.2 to 37.8)	40.7	(40.2 to 41.2)	41.6	(41.3 to 41.9)
Healthy GWG	0.1	(−1 to 1.2)	0	(−0.2 to 0.2)	0	(−0.2 to 0.2)
Inadequate GWG	0.8	(−0.4 to 2)	−0.1	(−0.7 to 0.5)	0	(−0.3 to 0.3)
Excessive GWG	ref	–	ref	–	ref	–
Normal weight (BMI < 25 kg/m2)	0	(−0.9 to 0.9)	0	(−0.3 to 0.3)	0	(−0.2 to 0.2)
Overweight (BMI 25 to 30 kg/m2)	0.2	(−0.8 to 1.2)	0.1	(−0.2 to 0.4)	0	(−0.2 to 0.2)
Obese (BMI ≥ 30 kg/m2)	ref	–	ref	–	ref	–
Takes folate (≥400 mg) (vs. not)	−0.2	(−0.9 to 0.5)	0	(−0.2 to 0.2)	0	(−0.1 to 0.1)
Some moderate-intensity exercise	0.8	(0 to 1.6)	0.2	(0 to 0.4)	0	(−0.1 to 0.1)
Frequent moderate-intensity exercise	−0.1	(−1.5 to 1.3)	0.1	(−0.2 to 0.4)	0	(−0.2 to 0.2)
No moderate-intensity exercise	ref	–	ref	–	ref	–
2 to 4 hours of television	0.5	(−0.6 to 1.6)	0	(−0.3 to 0.3)	0.1	(−0.1 to 0.3)
< 2 hours of television	0.3	(−0.8 to 1.4)	0	(−0.3 to 0.3)	0.1	(−0.1 to 0.3)
4+ hours of television	ref	–	ref	–	ref	–
Quit drinking during pregnancy	−0.4	(−1.1 to 0.3)	−0.2	(−0.4 to 0)	0.1	(0 to 0.2)
Quit drinking prepregnancy	−0.8	(−2.5 to 0.9)	−0.4	(−0.7 to −0.1)	0	(−0.2 to 0.2)
Never drank	0	(−0.9 to 0.9)	−0.1	(−0.5 to 0.3)	0.2	(−0.1 to 0.5)
Still drinks	ref	–	ref	–	ref	–
Quit smoking during pregnancy	0.5	(−0.5 to 1.5)	0	(−0.3 to 0.3)	0	(−0.2 to 0.2)
Never smoked	0.3	(−0.6 to 1.2)	0.1	(−0.2 to 0.4)	0	(−0.2 to 0.2)
Still smokes	ref	–	ref	–	ref	–
Eats 5 fruit/veg a day (vs. not)	0	(−0.7 to 0.7)	−0.1	(−0.3 to 0.1)	0	(−0.2 to 0.2)
Eats ≥ 1 serving of oily fish weekly (vs. not)	−0.1	(−0.7 to 0.5)	0	(−0.2 to 0.2)	0	(−0.1 to 0.1)

BMI, body mass index; CI, confidence interval; GWG, gestational weight gain.

Models further adjusted for infant sex, maternal age, maternal height, gravidity, ethnicity, whether the mother has a partner, maternal education, socioeconomic index, private/public maternity care, risk of depression, and stress score.

distribution is larger than those seen for fat-free mass and length, relative to their respective means and variances. This could be reflecting that newborn fat mass is more modifiable than infant fat-free mass and length, which are under stronger genetic control.[49,51,54,55] Consequently, we suggest that the nature of the GWG-fat mass relationship, which would have perhaps been missed using methods other than quantile regression, is reflecting a pathogenic effect of unhealthy GWG on fetal fat mass accumulation; whereas the reduction in fat-free mass associated with healthy GWG is just reflecting the functional relationship between the two variables.

Although maternal smoking is a long-recognized determinant of infant length and total mass, very few studies have investigated the associations of maternal smoking with newborn fat and fat-free mass. Lindsay *et al.*[56] ($n = 129$) and Spady *et al.*[57] ($n = 78$) found that maternal smoking was associated with decreased fat-free mass and length, but not fat mass. A larger ($n = 916$), more recent study also found that neonates exposed to smoking throughout the pregnancy had lower fat-free mass.[58] Our results are consistent with these.

Quantile regression also allowed us to observe that the highest centiles of fat mass were lower among infants born to women who never drank compared with women who were still drinking during pregnancy. It is important to note that the reported amount of alcohol being drunk by the latter group was low. Further, there was no appreciable difference in infant outcomes between women who were still drinking at recruitment and those who reported quitting before pregnancy. It thus seems likely that the observed association between never drinking and outcomes is at least partly explained by other factors experienced by this relatively small group of women (10% of sample).

Similarly, quantile regression revealed that the highest centiles of fat mass were also lower among infants born to women who engaged in frequent moderate-intensity exercise early in the pregnancy. This conflicts with a recent finding[59] in a sample of 826 mother-neonate pairs where physical activity in early pregnancy was not associated

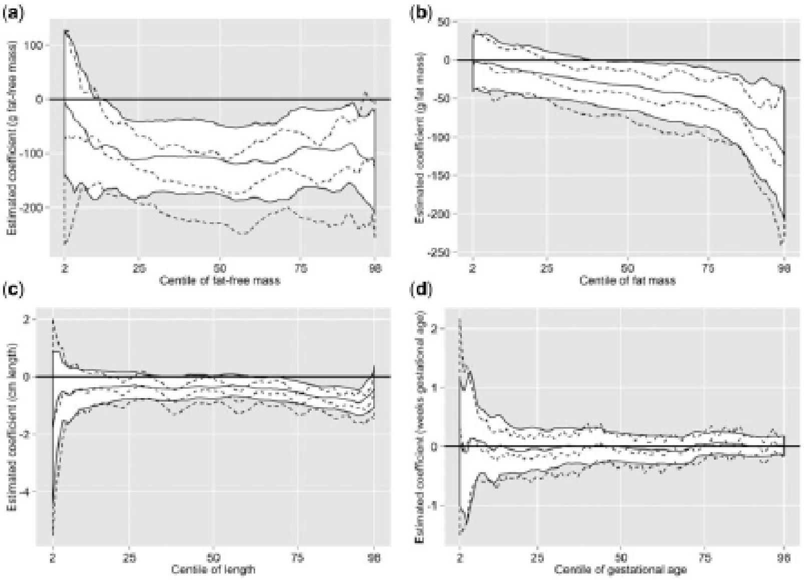

Figure 1. Healthy gestational weight gain. In each of the four plots above, the enclosed white space depicts the fully adjusted regression coefficients and 95% confidence intervals (values on the y-axis) for healthy (versus excessive) gestational weight gain at each centile (x-axis) of the dependent variable. The dashed lines similarly reflect the crude estimates and 95% CIs. Panel a shows that almost the entire distribution of fat-free mass is shifted to the left (towards smaller values) among infants born to women with healthy gestational weight gain (versus not), whereas Panel b shows that the right tail of the distribution of fat mass is being pulled in, with little change in the left tail of the distribution.

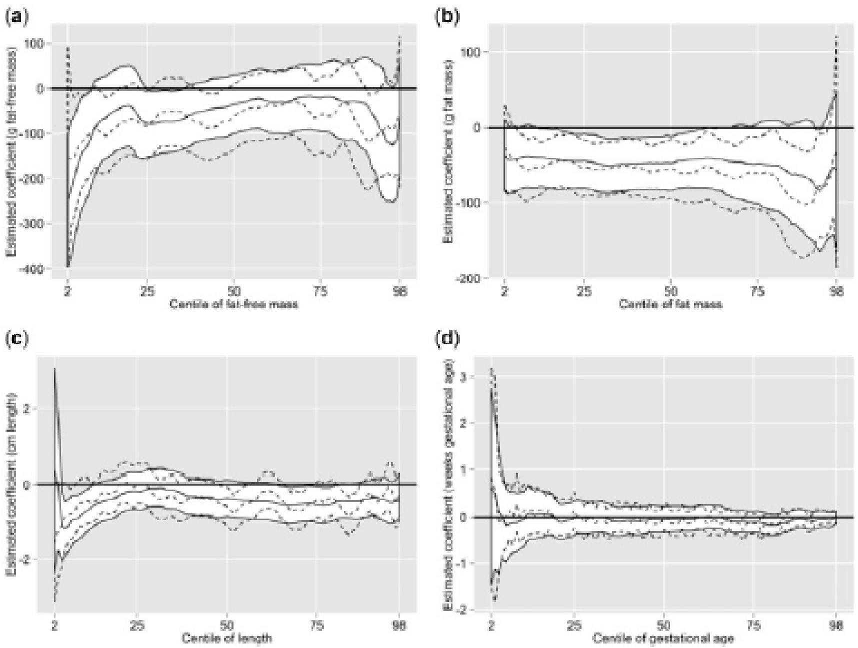

Figure 2. Maternal pre-pregnancy normal weight. In each of the four plots above, the enclosed white space depicts the fully adjusted regression coefficients and 95% confidence intervals (values on the y-axis) for maternal pre-pregnancy normal weight (versus obese) at each centile (x-axis) of the dependent variable. The dashed lines similarly reflect the crude estimates and 95% CIs.

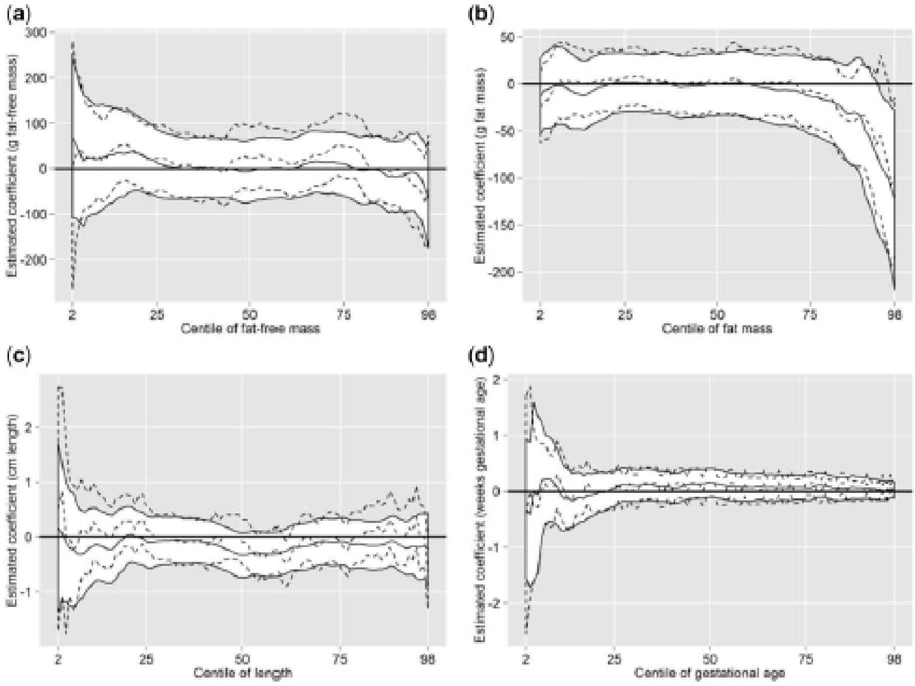

Figure 3. Frequent moderate-intensity exercise. In each of the four plots above, the enclosed white space depicts the fully adjusted regression coefficients and 95% confidence intervals (values on the y-axis) for frequent (versus not) moderate-intensity exercise at each centile (x-axis) of the dependent variable. The dashed lines similarly reflect the crude estimates and 95% CIs.

with fat mass, fat-free mass or birthweight. However, this study did not look at the tails of the fat mass distribution, and a difference like the one we observed could have been obscured in a comparison of mean fat mass values. While we caution against over-interpreting this result, it is encouraging to think that frequent, moderate-intensity activity might help reduce the prevalence of babies born with a very high amount of fat mass. Further, we found no evidence that exercise was associated with reduced fetal growth (reflected in birth length) or gestational age, which might help reassure everyone that moderate-intensity exercise during pregnancy is in fact safe.

Strengths

This analysis uses data from one of the largest studies of newborn body composition[60] measured with a reference method.[61] Further, the population-based nature of the study allowed us to estimate the independent associations of a variety of healthy and unhealthy behaviours in a more representative sample than is often possible.

Our use of quantile regression yielded insights that would not have been apparent with multiple linear regression. Binary or multinomial logistic regression is another common alternative for looking at the ends of a distribution, but when the outcome is inherently continuous in nature (e.g. mass), one must first impose one or more cutoffs. The logistic regression model treats any set of two values falling on different sides of a cut-off as similarly different, regardless of the distance between two values on the real number line. Thus quantile regression allows us to investigate the influence of a predictor on the tails of an outcome's distribution while still respecting the underlying continuous nature of that variable. The potential value of quantile regression for epidemiologists has been highlighted by Beyerlein,[62] and quantile regression has been previously applied to anthropometric studies across the life course.[63]

The ideal expression of infant body composition has been previously debated.[64] It is common to focus on percentage fat mass,[65] but this has limitations.[66] Ratio measures, generally, have poor statistical properties.[67] Additionally, because fat mass appears in both the numerator and the denominator of percentage fat mass, increases in absolute fat mass become less obvious as percentage fat mass increases. Further, focusing on fat

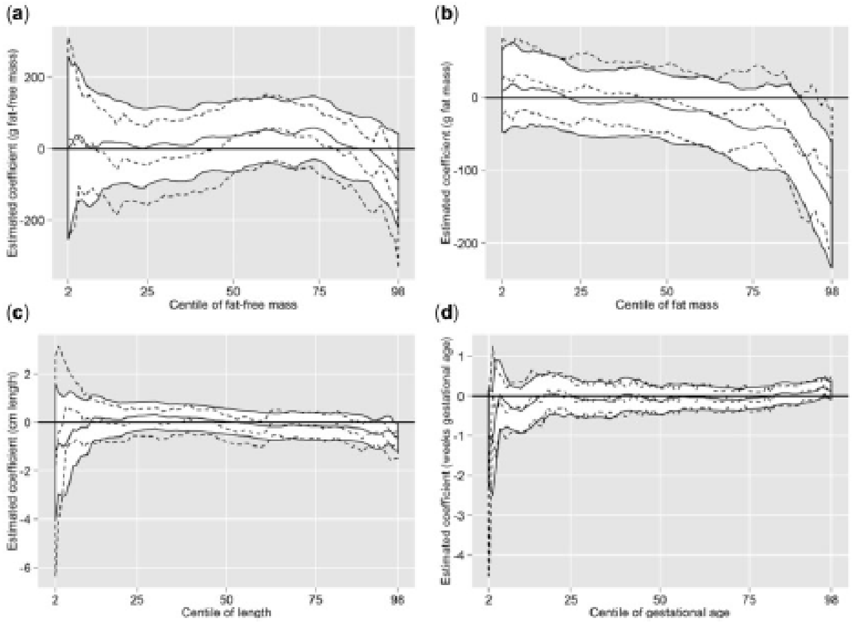

Figure 4. Never drank alcohol. In each of the four plots above, the enclosed white space depicts the fully adjusted regression coefficients and 95% confidence intervals (values on the y-axis) for never drank alcohol (versus still drinking) at each centile (x-axis) of the dependent variable. The dashed lines similarly reflect the crude estimates and 95% CIs.

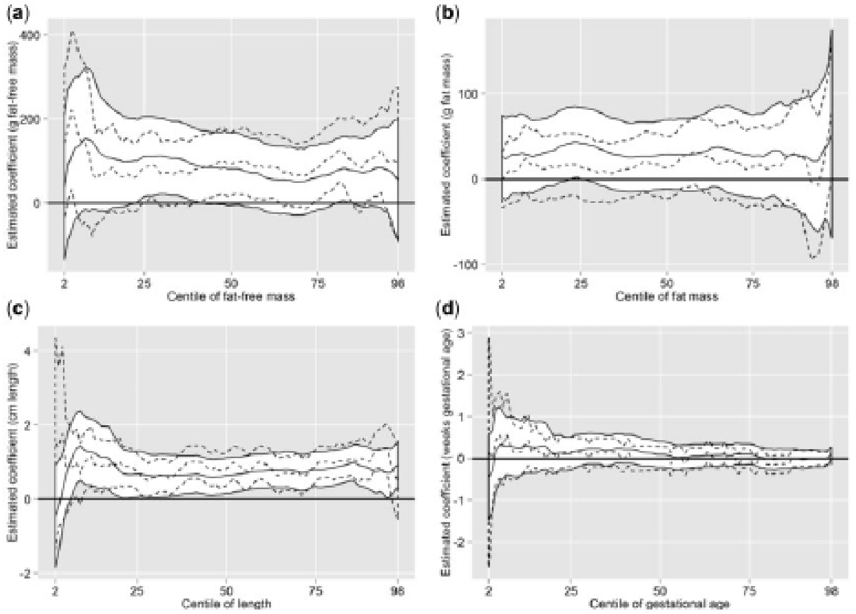

Figure 5. Never smoked. In each of the four plots above, the enclosed white space depicts the fully adjusted regression coefficients and 95% confidence intervals (values on the y-axis) for never smoked (versus still smoking) at each centile (x-axis) of the dependent variable. The dashed lines similarly reflect the crude estimates and 95% CIs.

mass can lead us to ignore the fat-free mass,[65] which is often just as important to consider.[68,69] Finally, though the supposed value of calculating percentage fat mass is to arrive at a measure that is normalized for overall body size, percentage fat mass is typically correlated with height, which was true in our sample (results not shown).

Contrary to common practice, we did not restrict the sample to term infants, nor include gestational age in our models of body composition or birth length. Because gestational age does not precede any of the maternal lifestyle factors we have investigated, it cannot confound associations between lifestyle factors and birth size. It could, however, mediate the influence of lifestyle on birth size, and thus adjusting for it could lead to collider bias when gestational age and birth size share other unmeasured causes. This issue has been previously described by Wilcox, Weinberg and Basso.[70] Similarly, we also did not adjust models of fat and fat-free mass for length, since it is likely that these will share unmeasured causes that again could confound associations between lifestyle factors and infant body composition once length is adjusted for.

Limitations

The main weakness of this research is that it is a secondary analysis of data collected with an observational study design. There is thus considerable potential for meaningful confounding and selection bias. These were hopefully minimized by thoughtful selection of covariates based on theoretical grounds. It is worth noting that models were not modified to better fit the data or in light of any preliminary analyses.

There were missing data for both outcomes and predictors in the sample. We used multiple imputation to estimate parameters under an assumption of missing at random (MAR), conditional on other observed covariates, which is more defensible than the assumption that data were missing completely at random (MCAR).[71] Our imputation models included the outcomes, which is recommended when there are missing values in the predictors.[72] However, we should note that the majority of missing values for body composition were because the PEA POD was not yet available on site, and thus those values are likely MCAR. Thus the main value of multiple imputation in this analysis is an increased sample size and a consequent increase in power to detect effects than had we instead used the complete case analysis, or an impute-then-delete approach.[73]

Most of the data on maternal lifestyle factors were based on self-report and are likely measured with considerable error. This was particularly true for the dietary data, which were based on questions about the consumption of nine selected food items rather than more established methods for dietary assessment, and so our ability to predict infant body composition from the dietary data was likely quite poor from the outset. Similarly, the survey question about moderate-intensity exercise, though consistent with the Centres for Disease Control (CDC) and World Health Organization (WHO) definitions, is quite broad. Importantly, useful information on gestational diabetes mellitus (GDM) was lacking on over two-thirds of the sample and was not considered in this analysis. However, whereas a GDM diagnosis can impact on subsequent lifestyle factors later in the pregnancy, it could not have affected the early/pre-pregnancy factors we have considered here, and thus could not have confounded the associations we report.

Conclusion

Despite its clear role in the developmental origins of health and disease, little is known about maternal influences on infant body composition. Taken as a whole, this analysis suggests that supporting healthy maternal lifestyles could reduce the risk of excess fat accumulation in the offspring, without increasing the risk of low body fat or adversely affecting fat-free mass development, length, or gestational age. We suggest that future evaluations of maternal lifestyle interventions include more direct infant body composition measures, and use quantile regression to analyse the subsequent data. The use of quantile regression led to insights that would not have been apparent in the data if using more commonly applied linear or logistic regression models.

Supplementary Data

Supplementary data are available at *IJE* online.

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Conflicts of interest: None.

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Appendix 4: Funding for PhD

The Cork BASELINE Birth Cohort Study is funded by the National Children's Research Centre, Ireland. Hazel A Smith is a PhD student had received funding by the National Children's Research Centre, Ireland for the first two years of her studies. Hazel was awarded €1,000 from the College of Medicine and Health Doctoral Student Bursaries, University College Cork to present results from her PhD at international conferences.