Parent-Child Reciprocity in Infant Feeding and Infant Weight Development: Bio-Psycho-Social Interactions

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Declaration

I, Kristiane Tommerup, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Abstract

Rapid weight gain in infancy (RWG) is a risk factor for overweight in childhood and adulthood. Formula feeding (FF) is a hypothesised cause, although mechanisms are unclear (e.g. due to feeding from a bottle or the nutritional content of formula milk). Emerging evidence indicates biopsychosocial interactions between parental feeding and child weight, but few studies have examined infant feeding modality (IFM) and weight in the critical first year of life. Part one of this thesis (Studies 1-3) triangulates epidemiology and the twin design to examine biopsychosocial interactions in a population-based cohort of n=2404 British twins born in 2007 (Gemini). In Study 1, infants fed with combinations of breastfeeding and FF, compared to being exclusively breastfed (EBF), had steeper weight gain trajectories across the first year of life. Both FF infants and those breastfed from a bottle showed steeper weight gain than those EBF from the breast, implicating bottle-feeding as a potential mechanism in RWG. The weight gain of twins discordant for IFMs did not differ and pointed towards potential reciprocity in infant feeding decisions: twins fed with more bottle or formula were smaller than their co-twin in early infancy. Study 2 explored reciprocity using bi-directional epidemiological analyses and twins discordant for IFMs. Slower early weight gain, and maternal concern for slow weight gain, predicted the introduction of formula milk. Study 3 explored whether FF is responsive to children's genetic liability towards slow early weight gain (i.e. gene-environment correlation), and whether breastfeeding buffers the expression of genetic influence on RWG (i.e. gene-environment interaction). No evidence of gene-environment interplay was found. The second part of this thesis (Study 4) describes the development of BRIGHT (Baby Responsive Intervention for Growth & Health Tracking), a digital intervention aiming to reduce RWG among FF infants by supporting responsive bottle-feeding, integrating insights from Studies 1-3.

Impact Statement

In England, nearly 1 in 4 children (22.4%) are affected by overweight or obesity by age 4-5, highlighting the early years as a critical period for the establishment of healthy weight development. Whilst rapid weight gain in infancy (RWG) has been identified as a risk factor for future overweight, there is a dearth of research exploring bio-psycho-social mechanisms which contribute towards RWG. In part one of this thesis, I triangulated bidirectional epidemiology with the twin design to highlight bidirectionality between IFMs and infant weight development. Findings from these studies may advance theory, clinical practice, and public policy in several ways. First, formula milk supplementation was, in part, a behavioural response to slower weight gain and maternal concern for slower weight gain in early infancy. Health care practitioners (HCPs), public health intervention developers, and policymakers seeking to support breastfeeding efforts or best-practice bottle-feeding practices would benefit from acknowledging such parent-child reciprocity. For example, if there is no sign of clinically relevant weight faltering and the caregiver wishes to continue breastfeeding, reassuring caregivers about their infant's weight gain may be an important strategy to support continued breastfeeding. Moreover, tailored approaches to infant feeding support may be more effective than 'one size fits all' strategies that do not address *child-driven* barriers to feeding behaviours. Caregivers will also benefit from these findings as better acknowledgement of parent-child reciprocity in clinical practice and feeding guidance may help reduce stigma – and resulting guilt – that surround formula feeding. The second key finding was that formula feeding was associated with more RWG in the period after its introduction, and that bottle-feeding (rather than or in addition to the nutritional content of formula milk) may be a key mechanism. Caregivers, infants, and HCPs would benefit from this information as interventions targeting bottle-feeding practices and behaviours could offer an important and unexploited opportunity to reduce RWG in formula fed infants. Moreover, the current lack of reputable bottle-feeding guidance available to caregivers may be detrimental for infant weight development as it leaves caregivers without adequate tools when introducing formula milk. The current thesis did not find evidence of GE interplay between IFMs and RWG – however, my study points to numerous opportunities for further studies to further explore potential GE interplay in infancy. In line with the findings from part one of this thesis, I presented the prototype for BRIGHT (Baby

Responsive Intervention for Growth & Health Tracking), a novel digital intervention to promote healthy growth by supporting responsive bottle-feeding practices amongst formula fed infants, co-developed with caregivers. Caregivers, infants, HCPs, and third sector organization's (e.g. charities and NGOs seeking to support caregivers by providing reputable bottle feeding guidance) will benefit from this comprehensive, and evidence-based package of formula feeding guidance, which is not currently available through reputable sources in the UK. BRIGHT has been presented at two international conferences, where findings were disseminated to researchers and policymakers. BRIGHT has also created a large network of international collaborators who have benefited from shared knowledge and resources and has supported 5 MSc Health Psychology research dissertations and 1 research placement at UCL.

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Abbreviations

RIWG: Rapid Infant Weight Gain

RWG: Rapid Weight Gain

IFMs: Infant Feeding Modalities

EBF: Exclusive Breastfed

PFPs: Parental Feeding Practices

BMI: Body Mass Index

Change z-score: Change in weight for age z-score

DT: Discordant Twin

GE: Gene-Environment

PRS: Polygenic Risk Score

LMMs: Linear Mixed-Effects Models

BRIGHT: Baby Responsive Intervention for Growth & Health Tracking

PBA: Person Based Approach for Intervention Development

APEASE: Acceptability (A), Practicability (P), Effectiveness (E), Affordability (A), Side-effects (S), and Equity (E)

TOC: Table of Changes

PPIE: Patient and Public Involvement and Engagement

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Chapter One. A Review of The Literature on Infant Feeding and Infant Weight Development for Part One of Thesis

1.1 Epidemiology of Childhood Obesity and Weight Development in the Early Years

Over the past four decades, the global number of children living with overweight and obesity has risen from 11 to 124 million, representing a near 10-fold increase.[1] Individuals who develop overweight or obesity in childhood are more likely to have obesity in adulthood[2], and carry a higher risk for developing type II diabetes[3], metabolic syndrome[4], and cardiovascular complications.[5] In the UK, the most recent data from the UK National Child Measurement (NCMP) programme from 2022/2023 reports that nearly 1 in 4 children have developed overweight or obesity (22.4%) by the time they enter Reception Year at age 4 to 5.[6] This figure increases to 36.6% for Year 6 children at age 10-11. Moreover, the NCMP data highlights widening socioeconomic gaps in obesity prevalence. In 2022/2023 reception children living in the most deprived areas were approximately twice as likely to have developed obesity (12.4%) than those living in the least deprived areas (5.8%). Hence, the development of adiposity in the early years of childhood is not distributed equally across the population. Children living in deprivation show starker increases in their obesity prevalence than those living in affluent areas. Given the consistently high prevalence and these stark social gradients, efforts to reduce the early development of adiposity, particularly amongst the populations most affected, is a national priority.[7]

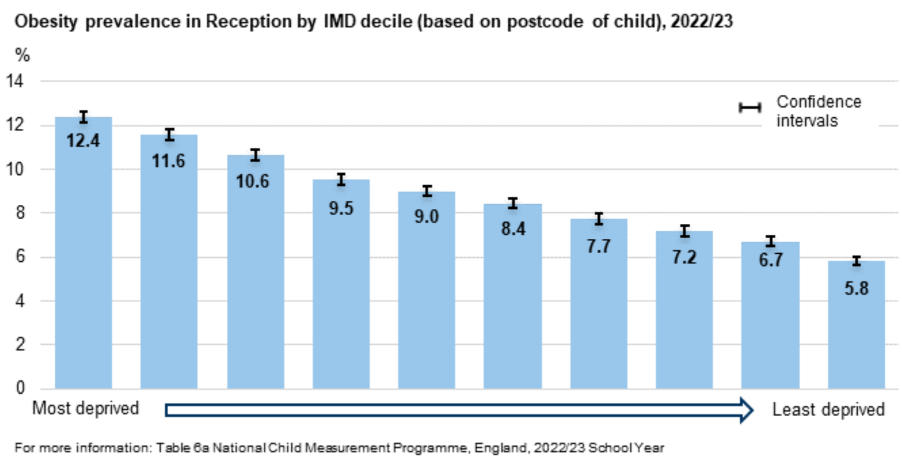


Figure 1.1 Prevalence of children living with obesity in Reception by Index of Multiple Deprivation decile (postcode) in 2022/23 obtained from the NCMP[6]

With nearly one in four children entering school with either overweight and obesity, it is crucial to look to early childhood and understand the factors which contribute to the development of excess adiposity in early life. Moreover, the development of excess adiposity, or excess body fat, in early childhood appears to be particularly problematic due to the strong tracking of overweight from early childhood into later adolescence, and even adulthood. Within a recent longitudinal cohort of ~50,000 German children, nearly 80% of 3-year-olds with a Body Mass Index (BMI) in the obese range ($\geq$98th centile) continued to have overweight or obesity in late adolescence.[8] Moreover, there is a substantial body of literature highlighting associations between infant weight development and future overweight and obesity, as described in this literature review. Taken together, the early life period or the first 1,000 days – from conception to age 2 - is critical in establishing healthy weight patterns across the life course. A health equity approach would also argue that interventions must target the systemic exposures which contribute to adiposity – given the strengthening social gradient of childhood obesity. However, to date, less research and policy level interventions and efforts have sought to understand the complex web of factors which contribute to adiposity in the early years period – and how these might be targeted to support healthy growth from early infancy.[9]

In sum, the high population wide prevalence, increasing social gradients, and demonstrated relations between early weight development and later adiposity, point to the first 1,000 days as a critical period for interventions and public health policy to promote healthy weight development across the life course.

1.1.1 Rapid Infant Weight Gain

Measuring and defining 'healthy' weight gain across the first 2 years of life is comparatively more complex than in later childhood and adulthood. Weight gain is highly variable across developmental periods of infancy – with infants experiencing 'growth spurts' where speed of weight gain is faster than in other periods of infancy. Therefore, infant growth is measured and evaluated according to specific age and gender of the child - using growth or centile charts. Growth charts (percentile curves showing the distribution of sex-stratified height and weight measurements in children) used for early childhood have been constructed using large data sets of healthy children. In this way, they provide a reference of 'healthy' growth, through which the growth of an individual infant can then compared and assessed.

In the UK, the UK90 growth reference charts were first developed to represent 'healthy' growth across infancy (0-2 years) in 1990.[10] These were constructed using weight data from British children collected in the 1980s who were both breastfed and formula fed.[10] These initial growth reference data simply described the growth of a large sample of representative children, and not a selected sample of healthy infants who represent growth under optimal circumstances. Hence, in 2006 the reference data were replaced by the World Health Organisation (WHO) Child Growth Standards to represent growth under selected optimal conditions.[11] These standards both expanded beyond British children to include children from six participating countries (Brazil, Ghana, India, Norway, Oman, and the United States), and focused on exclusively or predominantly breastfed children (n=8,500). Critically, the sample was restricted to infants who met a range of inclusion criteria including absence of health or environmental constraints on growth, adherence to breastfeeding recommendations, absence of maternal smoking, absence of significant morbidity, and single term birth. These growth standards are represented on sex-specific growth charts from 2 weeks of age. In practical terms, a male or female infant's weight (in kg) is plotted against their age to identify their weight-for-age standard deviation score (z-score) equivalent centile position. Centile lines from 2 weeks of age up to 5 years of age are displayed (e.g. the 75th percentile) to allow visual assessment of tracking, acceleration, or slowing in the speed of an infant's weight gain over time, using repeated weight measurements. When a subsequent weight measurement is taken and plotted, an important observation is whether that infant remains within their centile space and or crosses upwards or downwards through the adjacent centile lines – e.g. moving from the 75th to above the 91st centile space (or vice versa). Such upwards centile crossing, where an infant crosses at least two centile lines upwards (or one full centile band) within the first 2-years of life, has been proposed as a key indicator of 'risky' growth. Rapid Infant Weight Gain (RIWG) is therefore defined as the upward crossing of one centile band, equating to an increase in weight-for-age z-score of > 0.67.[12] Current guidance therefore proposes that optimal infant weight gain is tracking of weight within the same centile space from birth to two years of age – i.e. within the 50th to 75th or 9th to 25th centile. Conversely, downward centile crossing might indicate weight faltering which pose separate risks for health and development in future childhood[13,14] – it will not be discussed or explored in detail given the focus of the current thesis on early childhood obesity prevention.

1.1.2 Rapid Infant Weight Gain: Interplay of Influences across the TEAM-ECD Model

Weight development across the life course is a complex phenomenon. The early emergence of overweight and obesity is influenced by a complex set of factors which span from individual genetic factors to the home environment, to wider policies and societal systems which can influence behaviours and biology. As such it is important to draw on comprehensive frameworks that highlight the numerous layers of the individual, home, and social environment which influence early child development.[15] Ecological models, first developed by Dahlberg et al, such as the Total Environment Assessment Model for Early Child Development (TEAM-ECD)[16] developed for the World Health Organization's Commission on the Social Determinants of Health, draws on developmental psychology, biological embedding mechanisms, and the sociology of the early home environment. Hence, it considers how factors inside of the individual child (i.e. genetic factors, early infant weight development, infant temperament) as well as the home (i.e. economic resources, parenting styles, social support) and environment beyond the family (i.e. social norms, community resources, and cultural settings) account for variation and perhaps inequalities in child health and development.

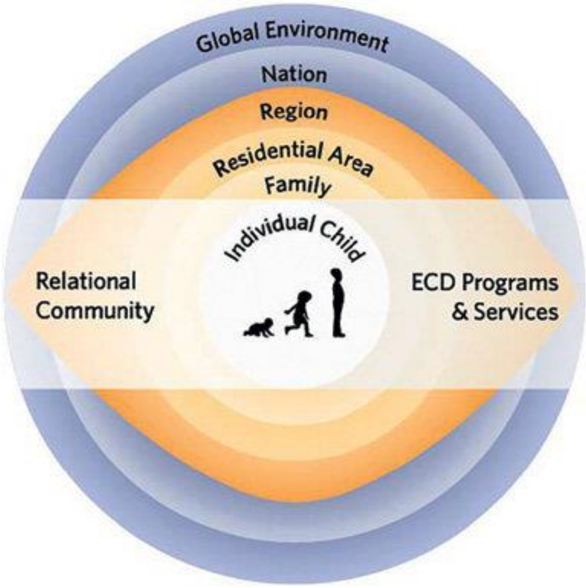


Figure 1.2. Total Environment Assessment Model for Early Child Development (TEAM-ECD) obtained from Irwin e tal[16]

In line with this model, it is not only imperative to consider how each of these layers might influence child health individually, but also to consider how they might be interconnected in their impact. The individual, family, and even residential area layers of influence are bi-directional in nature. For example, the family and residential area level might interact in that children born into lower economic (residential area level) resources may be fed with more pressuring and unresponsive feeding practices (family level) as families cannot afford a range of healthier foods or food waste and are therefore encouraged to eat what is put in front of them at any given time. However, given their complex nature most studies exploring determinants of infant weight development have focused on individual layers of influence in isolation – such as infant feeding methods at the family layer (summarised in section 1.2 of this review) and genetic influences at the individual child layer (summarised in section 1.3 of this review). Moreover, it is worth highlighting that more rigorous methodologies (e.g. experimental or genetically sensitive designs) are often needed to disentangle these layers of influence and explore such interactions as compared to traditional observational designs. To date, very few studies have explored possible bi-directional influences across the TEAM-ECD model in relation to infant weight development. The aim of this thesis is to measure these unexplored interconnections between the layers of the TEAM-ECD framework where possible. Moreover, this framework will be used to guide the present literature review – highlighting the individual layers of the TEAM-ECD model and interactions between layers which have been given less attention when studying the diverse factors that lead to adverse infant weight patterning. Together, applying this framework to the current literature review, and thesis more generally, will allow for a more rigorous, balanced, and nuanced understanding of how individual characteristics, the home environment, and the wider social environment coalesce to influence infant weight development. This model will enable the current thesis to highlight how biopsychosocial mechanisms interact to shape weight development and infant feeding reciprocity in the early years period.

1.1.3 Rapid Infant Weight Gain and Overweight and Obesity in Later Life

There is a substantial body of literature demonstrating observational associations between upward centile crossing, or RIWG, and greater risk of overweight and obesity in later childhood and adulthood. [12,17–19] A systematic review of 21 observational studies from a range of high, middle and low income countries showed that infants who experienced RIWG were at a 3.6 times greater risk (95% CI: 2.59 – 5.17) of later obesity.[18] Crucially, when RIWG was experienced before the age of 1, a 4.12-higher risk (95% CI: 1.83 – 9.28) of experiencing obesity in later life was shown. Therefore, it is particularly important to understand drivers of RIWG within the first year of life itself. Moreover, subgroup analyses indicated that associations were stronger for overweight and obesity in childhood (<18 years of age) (pooled OR = 4.16, 95% CI: 3.26–5.32; n=11) than in later adulthood ($\geq$18 years of age) (pooled OR = 2.02, 95% CI: 0.93–4.36; n=3), however the number of studies considering weight outcomes in adulthood was far less than those considering weight outcomes in childhood. In a more recent review, 45 out of 46 studies showed significant and positive associations between RIWG, at varying stages of infancy, and later risk of overweight or obesity.[19] Similarly, across a wider 282 studies evaluating evidence for various early life risk factors in childhood obesity beyond RIWG, it was reported that RIWG was one of the most consistent predictors of future overweight and obesity.[19] Finally, although links between RIWG and overweight and obesity in later life have been most commonly studied using BMI, studies with more detailed anthropometric measures such as fat mass index, have also shown associations between greater fat mass in early life and overweight in later life, suggesting that the association between RIWG and later BMI represents a tracking of fat mass and not simply lean body mass or height.[20] However, it is important to note that infants born at low birthweight and early are more likely to show RIWG or to 'catch-up' on their weight development by gaining weight more quickly than healthy birthweight infants.[21,22] Such RIWG may carry neurodevelopmental benefits for infants born pre-term or at a low birthweight.[23] Hence it is important to consider the health and weight implications of RIWG in the context of an infant's birthweight and gestational age. However, in large observational studies where birthweight and gestational age has been statistically adjusted for, associations between RIWG and future risk of overweight remain, suggesting that gaining weight rapidly poses a real risk for future overweight, independent of birthweight or gestational age.[18,19] Crucially, RIWG is also not constrained to low birthweight infants. For example, the Baby Milk trial in the UK estimated that 40% of healthy birth-weight infants fed with formula milk (n=669) who were formula fed experienced RIWG.[24] Moreover, findings from the Baby Milk trial highlighted the high prevalence of excessive energy intake amongst formula fed infants. The infants in the trial received an average of 107 excess kcals per day, or 16% more than the WHO average requirement of 666 kcal/day[24]. Based on these insights, it has been proposed that the monitoring and prevention of upward centile crossing in *healthy birthweight infants* (93% of infants born in the UK)[25] alongside supporting appropriate milk feeding practices among formula fed infants, may be a promising strategy to prevent the early emergence of overweight and obesity in childhood at the population level. For instance, the most recent report from the Scientific Advisory Committee on Nutrition (SACN)[26] highlighted optimising infant weight patterning as a promising target for future obesity prevention efforts.

One key limitation to consider across this field of research is the variety of measures through which RIWG is measured and presented. RIWG is most often measured in the literature as either an infant's increase in weight-for-age z-score (as a continuous measure) or as having experienced an increase of >0.67 in change z-score (as a categorical measure) – which can identify between any two time points of interest within the first two years. However, it has been criticised that cross-sectional or single measures of RIWG, such as change z-score at one point in infancy, may not provide an adequately reliable representation of infant growth.[12] Studies have shown that infants can 'rebound' in their growth (i.e. cross downwards following an upward centile crossing) which is more common after 6-months of age.[27] Moreover, infant weight can fluctuate substantially across developmental periods. Therefore, taking a single weight measurement to generate weight for age z-score – say at 6-months – may not be a reliable reflection of their growth trajectory to that point in time. Particularly if they are experiencing a rebound or growth *spurt*. Hence, researchers have called for more repeated indices of infant growth – such as modelling growth trajectories – to better understand whether accelerated growth in infancy is reliably associated with adiposity in later life.[28] However, few birth cohorts, particularly those with extensive measures of the early home and feeding environment, have repeated measures of growth or weight across infancy due to the substantial resources required to collect such detailed data. Hence, the current thesis seeks to use repeated measures of weight gain to explore infant weight gain trajectories as the primary measure of rapid infant weight gain.

1.2 Infant Feeding Methods and Infant Weight Development

The promotion of healthy weight development in the first 1,000 days of life has been proposed as a promising means to reduce the early emergence of childhood overweight and obesity given the strong tracking of weight patterning from the early period.[26] Thus, it remains a crucial priority for research to better understand the key determinants of accelerated weight gain in infancy. Given the early emergence of overweight and obesity in the UK - the identification of very early risk factors for childhood obesity is particularly crucial. Moreover, RIWG has been shown to carry numerous implications for health and wellbeing outside of weight, such as later cardiometabolic risk[29]. A more rigorous understanding of early risk factors will help to shed light on how best to reduce the prevalence of RIWG across the population. Similarly, in line with comprehensive bio-social frameworks such as the TEAM-ECD model[16], it is important to consider how risk factors across numerous layers of influence – from the individual child to the home environment – might *interact* to place a child at risk of RIWG.

1.2.1 Infant Feeding Methods: History and Epidemiology

Infant weight development is strongly related to energy intake, more so than in any other period of life, with energy deposition as a percentage of total energy requirements decreasing from 40% at 1 month of age to 1-2 % from 12 months until mid-adolescence.[30] Therefore, to date, most of the research exploring behavioural and environmental risk factors for RIWG and the presence of overweight in later childhood have placed emphasis on infant feeding methods– consisting of exclusive breastfeeding (EBF), formula feeding, or a mixture of the two (at the family level of the TEAM-ECD model). Breastfeeding can occur either as drinking milk directly from the breast or drinking breastmilk which has been expressed into a bottle. In 2001, the WHO announced its recommendation that infants should be exclusively breastfed for six months and continue to breastfeed alongside the introduction of solid foods until 2 years, or beyond[31]. Although there is a lack of high-quality survey data to evaluate the uptake of this recommendation in the UK at present, the available data suggests low adherence to breastfeeding recommendations across the UK population. Although collected more than a decade ago, the most detailed source of data come from the Infant Feeding Survey undertaken in 2010 (n= 10,768 mothers). This survey indicates that 81% of women initiate breastfeeding after birth. Yet, rates of EBF drop to 24% at six weeks, while the rate of 'any' breastfeeding (indicating mixed feeding) was at 55% at six weeks.[32] At six months, which is the age at which the WHO recommends women exclusively breastfed their infants until, the rate of exclusive breastfeeding was at 1%, with 'any' amount of breastfeeding at 34%. These descriptive data suggest that a mere 1% of women were meeting the WHO guidelines on exclusive breast feeding in 2010, although it is of note that many women (55% at six weeks and 34% at six months) are using breastmilk in combination with formula milk to feed their infants during the milk-feeding stage. Moreover, low EBF rates at 6-months may reflect the introduction of solid foods rather than supplementation with formula feeding. More recent but less comprehensive data from NHS England and the Office for Health Improvement and Disparities (OHID) in 2022, indicate that 51.7% of women in England (n=480,999) partially or exclusively breastfed their infants at 6 to 8 weeks of age.[33] These data were collected through a reporting system set up to collect health visiting activity at the local authority level. These 'low' breastfeeding rates in the UK, both from 2010 and 2022, which are also present in many other mid and high-income nations[34], has led to a wealth of research and discourse investigating the potential implications of infant feeding methods on weight development both during and beyond infancy.

Whilst there has been substantial exploration of the association between breastfeeding and weight outcomes for children, particularly in later childhood, the literature has largely relied on observational methods. Whilst the breadth of these studies is important to consider, the limitations of observational data such as social and genetic confounding, as well as selection and measurement biases, may lead to overestimated or confounded associations between breastfeeding and child weight outcomes. Hence, it is crucial that these limitations are considered and alternative methodologies which can generate more robust associations between breastfeeding and weight development are applied prior to any claims of causal inference. First, sibling or twin studies can examine the association between infant feeding modality and weight through within-family comparisons that remove confounding from environmental factors shared by siblings (e.g. socioeconomic position).[35] However, there are only a handful of sibling studies that have sought to test these relationships. Furthermore, whilst randomised controlled trials (RCTs) would offer the highest quality form of evidence, they are challenging to undertake within this field as randomising infants to receiving formula milk or breastmilk is unethical given the emotive subject of breastfeeding as well as the benefits of breastfeeding outside of from weight development (e.g. infections within infancy).[36] Nevertheless, there have been a few RCTs of breastfeeding *promotion*, which aim to increase breastfeeding rates in an experimental group and measure implications for infant and child weight development. Interventions that succeed in increasing breastfeeding in the intervention group can provide useful insights into infant feeding outcomes under experimental conditions. However, each of these methodologies – observational, sibling studies, and experimental designs - carries their own inherent strengths and limitations, which need to be considered. The following review therefore seeks to explore the influence of infant feeding methods – or more simply, breastfeeding versus formula feeding – on infant weight outcomes across these methodologies. First, experimental designs are reviewed, then sibling studies, and then observational studies of unrelated individuals, with a consideration of their limitations and challenges regarding causal inference.

1.2.2 Causal Inference in The Relationship Between Breastfeeding and Child Weight Development

1.2.2.1 Experimental Findings and Trial Designs

Findings from RCTs and clinical controlled trials are often considered the gold standard of effectiveness research – as randomisation can address biases of social and genetic confounding, reverse causation, as well as some measurement errors and researcher biases. In the context of Infant Feeding Modalities (IFMs), randomising infants to be fed either through EBF or formula milk, regardless of their family's social standing or demographic characteristics, could help to clarify the extent to which breastfeeding influence child and infant weight outcomes independent of confounding from socioeconomic or genetic factors. Secondly, random allocation to IFMs could overcome self-section biases, whereby mothers who breasted are may be an inherently 'self-selected' subgroup who breastfeed as their infant is not experiencing weight faltering, expressing a challenging temperament, or expressing an avid or small appetite which leads to supplementation with formula milk. In this way, there may be numerous genetic, social, and individual infant characteristics that exaggerate or distort relations between feeding and growth which can best be controlled for and measured by experimental design, such as an RCT. However, it is not ethical to randomise infants to receive either breastmilk or formula milk given the wide-reaching health benefits of breastfeeding in the early years of childhood. Therefore, only rare circumstances over the past few decades have allowed for the relationship between breastfeeding and infant or child weight outcomes to be tested under truly randomised allocation. However, more studies have been able to explore the influence of a *breastfeeding promotion* trials on infant outcomes. In these studies, differences in weight development between infants receiving breastfeeding promotion, and those not, can be attributed to differences in breastfeeding rates that result from the intervention. Whilst limitations still need be considered here, and will be discussed, data from such trials can provide useful evidence regarding the influence of breastfeeding on infant growth, removing residual social confounding and self-selection effects that hinder observational studies of unrelated individuals.

In one unique RCT, Singhal and colleagues[37] examined data from a cohort of infants born pre-term in 1982-1985 (n=926) in the UK. As the benefits of breastmilk over pre-term specific formula had yet to be established among this group of infants, they were able to be randomised to receive varying types of milk from birth. These data were composed of two trials; the first compared 502 infants randomised to receive either expressed breast milk or formula designed for pre-term infants, enriched with protein and fat (pre-term formula; 2·0 g protein and 4·9 g fat per 100 ml versus term formula; 1·5 g protein and 3·8 g fat per 100 ml). The second trial compared 424 infants randomly assigned to receive either term formula or formula designed for preterm infants. These assigned milk diets were given until the infant weighted 2000g or was discharged home. Moreover, across both these comparison groups, social standing was equally distributed to control for confounding from socioeconomic positioning. Findings indicated that within the first trial, infants receiving pre-term formula (n=64) gained significantly more weight during hospitalisation (15.8 g/daily) than those given expressed breastmilk fed (14.0 g/daily) (n-66). In the second trial, there was no significant difference in daily weight gain between infants given term formula (n=44) (13.7 g/daily) versus pre-term formula (n=42) (14.9 kg/daily). However, when evaluating variations in child weight at a follow up at 13-16 years of age, no differences in weight outcomes were observable between the groups.[37] These findings offer experimental evidence that '*what*' an infant is fed – i.e. formula milk as opposed to expressed breast milk – might independently contribute to more rapid weight gain in infancy, yet these effects were not sustained into adolescence. However, it is crucial to note that the comparison was undertaken between breastmilk and enriched formula milk, raising the question of whether this significant difference would have presented when comparing breastmilk to regular formula milk. Moreover, the sample consisted exclusively of pre-term infants born at a low birthweight who are more likely to show RIWG or catch-up growth[22] thereby limiting generalisability to term, higher birthweight infants. Finally, this trial was undertaken ~40 years ago, raising the question of cohort effects and the influence of formula milk reformulation.

Another source of experimental data comes from breastfeeding *promotion* trials, where an intervention group, receiving promotion, education and/or guidance and support to raise rates of breastfeeding, is compared to a control group. Hence, these trials test the influence of breastfeeding promotion or support as opposed to breastfeeding behaviour in and of itself. Such trials have been undertaken in countries where breastfeeding rates or support is low, as in contexts containing high support already there would be little opportunity to increase breastfeeding rates through such intervention. In a recent meta-analysis of 35 of such trials, Giugliani and colleagues found that breastfeeding promotion interventions had no effect on weight z-scores [pooled effect: 0.03 (95% confidence interval: −0.06; 0.12] but led to a modest reduction in body mass index of the infants [z score mean difference: −0.06 (95% confidence interval: −0.12;0.00)].[38] Moreover, the influence of breastfeeding promotion could vary by the country and setting of delivery. For instance, one exclusive breastfeeding promotion intervention showing opposite effects in Burkina Faso and South Africa – as the intervention increased weight-for-height z-scores at 12 and 24 weeks in South Africa; yet led to lower weight-for-height z-scores at six months in Burkina Faso.[38] These findings might be attributed to the hypothesis that in low-income countries or those with limited access to clean water, formula feeding might result in reductions in weight due to increased risk of GI infections and practices like formula stretching which could impede growth. This is the opposite direction than what would be assumed in high income countries. However, this hypothesis was not supported by the systematic review as the impact of breastfeeding promotion interventions on growth from three studies in low-income settings were smaller than those other higher-income settings.

Nonetheless, the most important limitation to consider in relation to breastfeeding promotion trials is that these studies are merely measuring the influence of a breastfeeding intervention, rather than actual breastfeeding behaviour. Therefore, they are subject to the success of the intervention in increasing breastfeeding behaviour. Hence, there may be substantial formula feeding within the 'breastfeeding education' intervention group – which would dilute the effect of the breastfeeding promotion interventions. One landmark study, which is important to evaluate with consideration of this limitation, is the Promotion of Breastfeeding Intervention Trial (PROBIT) undertaken in Belarus between 1996-1997[39]. Prior to the trial, Belarus health bodies and organisations did not promote breastfeeding, resulting in very low breastfeeding rates across the population. However, in keeping with high-income countries, it carried a well-functioning healthcare system, sanitation systems (e.g. access to clean water) and child health outcomes. Therefore, this was a context in which a breastfeeding promotion trial could realistically increase breastfeeding rates in the population, alongside many of the country's characteristics being highly comparable to higher income countries like the UK, thereby allowing the effects on health outcomes to be examined and generalised outside Belarus. Mothers randomised to the intervention condition received breastfeeding promotion and support (n= 8865), whilst in the control condition mothers only received the usual care with standard infant feeding policies and support (n= 8930). As a result, infants from the intervention condition were more likely to be breastfed with any breastmilk at 12-months (intervention reference vs. control; 19.7% vs 11.4%; adjOR, 0.47; 95% CI= 0.32-0.69) as well as exclusively breastfed at 3 months, which was the public health guidance at the time (43.3% vs 6.4%; P<.001) as well as predominantly breastfed (51.9 vs 28.3%; adjOR, 0.28; 95% CI= 0.16-0.49). When evaluating infant growth across these conditions after the trial, the results did not show lower weight or less RIWG in the experimental breastfeeding education group as hypothesises. Regarding weight outcomes, there was no difference observed at 12-months between conditions (10,564 g vs 10,571 g) and further analyses of weight-for-age z-scores also showed no significant difference across the first 12-months of life.[40]Of note, both groups exceeded the WHO standards for infant growth. Moreover, in a longitudinal follow up of the PROBIT trial (n=13,557), the intervention group also did not show a lowered risk of obesity in adolescence.[41] In fact, the prevalence of overweight and obesity was significantly higher in the breastfeeding promotion group than the control group (BMI≥85^{th}; adjOR = 1.14, CI = 1.02 - 1.28). Taken together, these findings could suggest that when social patterning of breastfeeding behaviour is removed due to intervention randomisation, breastfeeding no longer shows marked benefits for weight development in infancy or in later life. However, once again, it is critical to consider the substantial overlap in breastfeeding and formula feeding across the two conditions – as 56.7% of PROBIT mothers in the ‘breastfeeding’ condition were using formula milk at 3-month of age. Therefore, it remains possible that the lack of effect may be due to intervention dilution, and stronger effects may have presented themselves under more strict comparisons between EBF and exclusive formula feeding if possible.

In response to these limitations, further analyses of the PROBIT trial have been conducted in attempt to remove the dilution brought on by the initial intention to treat analyses. For instance, a recent observation or ‘as fed’ analysis compared infant weight gain trajectories between PROBIT infants were breastfed (with any breastmilk) for 12 months or more with those breastfed for less than 12 months.[40] Infants breastfed for less than 12-months showed lower weight to 2-months of age, but then grew more rapidly – exceeding the weight of those were breastfed for >12-months by 6-months of age. Hence, longer durations of breastfeeding may have slightly favourable outcomes for weight gain trajectories in the first year of life when actual breastfeeding is considered as opposed to allocation to a breastfeeding intervention. Moreover, the small magnitude in differences across these groups may also reflect that many in infants in the breastfed <12-months comparison groups were also breastfed for an appreciable duration. Hence, more stringent comparisons of formula feeding versus EBF may have garnered more significant variations.

Taken together, findings from breastfeeding promotion trials have not shown marked evidence of breastfeeding promotion on more favourable weight gain trajectories.[38] It has therefore been put forward that removing social confounding may thereby remove the observational benefits of breastfeeding on infant weight development. However, as aforementioned, the consideration that breastfeeding and formula-feeding is highly diluted across experimental and control groups within these trials may hinder the extent to which any influence of IFMs on infant growth may be measured in the first place. It is therefore not possible to conclusively determine that the lack of influence of breastfeeding on infant growth in promotion trials is merely due to the removal of social confounding. It therefore may be useful to also look to quasi-experimental family designs to help answer the question of whether breastfeeding is robustly associated with favourable weight in infancy.

1.2.2.2 Twin and Sibling Designs on Infant Feeding Methods and Child Weight Development

Sibling studies have long been used as 'natural experiments' that allow researchers to make causal inferences from observed associations of human behaviour, particularly where randomisation trials are neither ethical nor feasible such as for breastfeeding. As siblings typically share many aspects of their early home environment – such as socioeconomic positioning of their family - comparing sibling pairs or twin pairs can substantially reduce the risk of bias from social patterning of behaviours often present in observational studies[42,43]. Hence sibling designs can help to clarify the extent to which IFMs drive infant weight patterning independent of exogenous aspects of advantageous social settings, such as greater access to healthcare and quality of diet in later childhood. Whilst siblings may share their early home environments to a large degree – other nonshared environmental factors may arise from the different timings or settings in which siblings are born.

The twin design, using within-twin pair comparisons, offers an even more robust method to control for the influence of shared environmental factors. As twins are usually reared by the shared caregivers, in the same household, and at the same time (in contrast to sibling comparisons) it allows for greater control over exogenous confounding present of observational comparisons between unrelated samples. Twin designs also offer a powerful methodology to disentangle the relative importance of genetic and environmental contributors to early weight gain, further described in section 1.3.1 of this review. Specifically, the twin design gives rise to opportunities to employ quasi-experimental methods to generate more causal inference insights – such as the discordant twin (DT) method.[35] The DT method involves comparing an outcome of interest – such as weight gain – within twin pairs who are discordant for (or treated differently for) an exposure of interest. The methods and benefits of DT designs are explained further in section 2.5.6 of Chapter Two. Crucially, sibling and twin comparisons offer a unique and critical opportunity to counter the confounding influence of social patterns in breastfeeding behaviour– as twins are reared in the same home and under the same social positioning. However, only a few sibling studies and only one DT study have been undertaken on the relationship between breastfeeding and child weight outcomes.

Looking across the limited sibling studies to date on this topic, there is little convincing evidence that the benefits of breastfeeding remain when shared environmental factors are better accounted for. For instance, using the National Longitudinal Survey of Youth (NLSY), Colen & Ramey[44] found that despite an identifiable association between breastfeeding and childhood BMI within an unrelated sample, when more stringent sibling comparisons were made in siblings discordant for IFMs these associations were no longer present (n= 1,773 children from 665 families). This was true of both BMI as well as a number of other health related outcomes, leading the authors to suggest that much of the beneficial effect of breastfeeding on childhood BMI might be due to the selection of breastfeeding behaviours in advantageous social environments. Similarly, a recent systematic review of six sibling pair studies undertaken on breastfeeding and the risk of overweight from the age of 2 onwards, confirmed this pattern of results.[45] Stronger associations between breastfeeding initiation and duration were present in unrelated samples where risk of confounding from social standing is greater.[45] Specifically, only 1 of 4 sibling-pair studies found significant associations between breastfeeding and childhood weight outcomes. In this subsample of discordant sibling pairs (n=488 pairs), the sibling having ever been fed breastmilk showed lower BMI z score (mean ± SE: –0.397 ± 0.176; $P < 0.05$) from 9-19 years of age as compared to their sibling never fed breastmilk. Moreover, in a more specific discordant twin subsample (n=44 pairs), which was able to control for difference in age and timing of siblings, the twin ever fed breast milk showed lower odds of obesity (OR =0.70, $P \geq 0.05$) than their co-twin never fed breastmilk, warranting further DT twin comparisons on this topic within infancy.[46] Nonetheless, across the studies to date, the breadth of sibling studies do not support causal associations between breastfeeding and weight outcomes in childhood. Moreover, it is important to note that the sibling design cannot rule out the presence of confounding from non-shared environmental factors which might vary across the twin pair – such as early infant growth, appetite, or random illness. These nonshared factor could influence between sibling and twin comparisons and need be considered carefully. Regardless, to date there have been no sibling pair or DT studies of the association between IFMs and weight patterning in the infancy period. Application of this design within the first year of life would be useful to understand whether breastfeeding influences RIWG independent of social and genetic confounding.

1.2.2.3 Observational Studies of Infant Feeding Methods and Child Weight Development

Given the ethical challenges of randomising infants to either receive breastmilk or formula milk and the limited availability of sibling and twin cohort studies, the literature investigating the association between infant feeding methods and weight outcomes largely relies on observational methods. The majority of this literature has focused on child weight outcomes; however a handful of studies have examined associations between breastfeeding and infant weight outcomes such as RIWG. As observational studies are overrepresented amongst the breastfeeding literature, it is critical to interpret their findings considering the inherent limitations of observational designs. Hence, the present literature review will present these findings alongside three critical biases that are difficult to eliminate under observational comparisons; i) measurement error in breastfeeding measures; ii) confounding from social patterning of breastfeeding behaviour; iii) reciprocity in the infant-caregiver feeding relationship. These findings and the presence of such biases will largely be explored in relation to IFMs and child weight outcomes, as the vast majority of the literature has considered child weight outcomes. However, observation results and their limitations regarding IFMs and infant weight outcomes will be presented where possible.

The Association between Breastfeeding and Child Weight Outcomes

Studies linking breastfeeding initiation and duration to child weight outcomes have produced varied results. A comprehensive meta-analysis of the available observational literature highlighted inconsistences in associations between breastfeeding and child weight - with 23 out of 49 (47%) prospective observational studies demonstrating a significant relationship between breastfeeding and decreased odds of childhood overweight.[19] Similarly, a recent review by Horta and colleagues of 159 studies[47], showed that, on average, breastfed infants were less likely to develop overweight or obesity (Pooled OR: 0.73, 95% CI: 0.71; 0.76). However, amongst the smaller subset of 19 studies of higher quality, as they were undertaken on larger cohorts and adjusted for confounding from socio-economic status and parental anthropometry, this association was weaker (OR: 0.85, 95% CI: 0.77; 0.93) such that breastfed children were 15% less likely to develop obesity than children fed with formula milk. Whilst the authors concluded that these more stringent results suggest that the protective effect of breastfeeding against later obesity is robust and not due to bias, it is important to still consider the inherent limitations *within* each of these observational studies themselves.

The first key limitation across the observational breastfeeding literature is measurement error in regard to operationalisation of breastfeeding behaviour. In their review, Woo Baidal and colleagues highlighted the inconsistency of breastfeeding definitions included studies in their review.[19] Moreover, recent reviews have pointed towards the inconsistent empirical measurements of infant feeding which requires urgent improvement.[48] Whilst some studies defined breastfeeding as EBF, other studies defined it as ‘any’ provision of breast milk, with varied durations and ratios of breast to formula milk provision within this single group. Hence ‘formula fed’ babies could be compared to a ‘breastfed’ baby who received highly varying proportions of breast to formula milk. This creates statistical noise and could dilute effect sizes. Moreover, in a recent analysis of peer-reviewed literature on breastfeeding undertaken (n=114 studies) over the past two decades, some descriptors of 'breastfeeding' were present in 68% of studies, however full definitions which distinguished the duration or ratio of breastmilk in the breastfeeding group were only offered in 28% of the studies.[49] The authors proposed that this heterogeneity limits comparability and interpretation of findings across the literature. For example, the true benefits of breastfeeding may be masked by comparing EBF to partially breastfed infants (who may have either received a large or small ratio of breast to formula milk if not clearly defined by the authors). Similarly, aggregations of direct use of expressed breastmilk, introduces the possibility that bottle-feeding per se (regardless of what is in the bottle) influences growth outcomes. It has been hypothesised that bottle-feeding, and its associated behaviours, allows for a greater capacity for controlling or unresponsive feeding behaviours from parents and less opportunity for the infant to direct the feeding process. [50] The resulting ambiguity of expressed breastfeeding stratification and definitions, may lead to biased effects of breastfeeding on growth outcomes. In response to these measurement biases, Azad and Colleagues[51] have called for greater clarity across domains of breastfeeding operationalisation, as highlighted by **Figure 1.2**. Clearer operationalisation of breastfeeding in future observational studies and interventions can help to better answer a number of questions which remain such as; i) the extent to which the duration, the length of breastfeeding provision, is beneficial for child weight outcomes ii) whether the exclusivity of breastmilk is necessary for optimal weight outcomes and iii) whether breastfeeding from the breast versus from a bottle leads to differing weight outcomes.

B) Non-bias inducing limitations (measuring different effects)

- ***Misclassification of breastfeeding exposures***—Standardized definitions have been proposed for breastfeeding research, but many studies do not apply them (Miliku & Azad, 2018). Ideally, studies should capture and distinguish the following:
  - Duration and exclusivity of breastfeeding
  - Nursing at the breast vs expressed HM (relative proportion of each; storage of expressed milk)
  - Perinatal feeding exposures in hospital
  - Introduction of complementary foods (both age and type/quality of food)
  - If partially breastfed: relative proportion of HM vs infant formula
  - If bottle fed (whether infant formula or HM): feeding style
  - If formula fed: variation in type of infant formula used (e.g. high/low protein, protein source and size, percentage carbohydrate from lactose, addition of pre/probiotics, lactoferrin, milk fat globule membrane, etc.)

Figure 1.3. Sources of heterogeneity in epidemiological studies of breastfeeding: Misclassification of Breastfeeding Exposures obtained from Azad and Colleagues[51]

The Association Between Breastfeeding and Infant Weight Outcomes

Overall, there have been fewer studies evaluating associations between infant feeding methods on weight outcomes in infancy as compared to later childhood. However, findings have shown similarly mixed results regarding the extent to which breastfeeding is associated with more optimal growth patterns. In keeping with findings of weight outcomes in later childhood, this heterogeneity has been argued to partly be a result of the significant discrepancy within and lack of clear operationalisation of breastfeeding exposures. Nonetheless, a few studies have attempted to investigate associations between breastfeeding and infant growth with more rigorous definitions of exclusivity and duration of breastfeeding and expressed breastfeeding (Johnson et al[52]; Durmus et al[53]; Azad et al[54], and Li et al[50]). First, using a binary measure of breastfeeding (breastfeeding with any breast milk, inclusive of expressed breastfeeding, for longer than 4 months versus never breastfeeding), the Gemini Twin Birth Cohort (n=4772) found that infants breastfed showed slower growth velocity or slower rate of growth (6.8%, SE = 1.3) than those who were never breastfed (N=1097 (23%)).[52] These findings suggest that any amount of breastfeeding (not simply exclusive breastfeeding) for more than 4 months carries benefits for less rapid growth in infancy. However, this binary categorisation of breastfeeding did not permit an exploration of the nuanced ways in which breastfeeding duration and exclusivity may impact infant growth. Expanding upon these results, a more recent population-based Dutch cohort (n=5047), examined both the duration and exclusivity of breastfeeding. Durmus et al compared rapid growth between i) by *duration* of any breastfeeding, and ii) between infants breastfed *exclusively* and partially breastfed for 4 months.[53] Rapid growth was defined by increases in weight for age z-scores between 3 and 6 months of age. Regarding duration, children who were never breastfed (B=0.17, 95%, CI: 0.10 - 0.24) or for <3 months (B=0.19, 95% CI:0.15- 0.24) had higher gains in weight z-scores between 3-6 months than those breastfed for 3-6 months, suggesting the potential benefit of breastfeeding beyond the first 3-months of life. Moreover, as compared to EBF infants, infants who were either never breastfed (B=0.24, 95% CI: 0.17 - 0.32) or partially breastfed (B=0.23, 95% CI: 0.18 - 0.27) until 4 months showed higher increase in weight z-scores then EBF infants between 3-6 months. However, breastfeeding duration and EBF was not associated with overweight and obesity at the age of 12-months, 24-months, or 48-month follow ups. These findings suggest the benefits of breastfeeding were not maintained by 12 months of age and may not show robust benefits for weight gain beyond the first few months of life. Finally, and crucially, it was not specified by the authors how infants fed expressed breast milk were treated in the sample, leaving important questions regarding the effects of feeding expressed breast milk on infant growth outcomes.

To address this limitation, Li and colleagues used the Infant Feeding Practices Study II (n=1899)[50] undertaken in the US to compare RIWG between bottle-fed babies fed with formula milk and bottle-fed babies fed with expressed milk. The authors proposed that this direct comparison between both milk type (formula vs. expressed breast milk) within a constant modality (bottle) helps to clarify whether it is the content of milk or the mode through which the milk is delivered which may be a mechanism leading to higher rates of rapid growth in formula-fed infants in much of the literature. The authors used the detailed feeding mode data available to stratify infants who were i) exclusively breastfed ii) exclusively expressed fed iii) expressed fed and formula fed iv) breastfed and formula fed v) breast fed and expressed fed and vi) formula fed exclusively. The more detailed stratification of mixed feeding demonstrated that regardless of the type of milk placed in the bottle, bottle-feeding appeared to promote rapid growth across the first 12 months of life. In other words, infants fed by formula milk (n=2016, weight gain/month= 71.25g, 95% CI: 56.03 - 86.47) and expressed breast milk (n=34, weight gain/m = 88.83, 95%CI: 13.19 - 164.47) gained more weight per month as compared to directly breastfed infants, controlling for several socio-demographic factors and infant characteristics. However, it is important to note the small sample of expressed fed infants (n=34) given the rarity in exclusivity of this feeding method. Moreover, within the 'mixed-feeding' groups, infants fed both expressed milk and formula milk only gained 37g more per month (95% CI: -5.06 - 79.42), infants fed breast and expressed breast milk gained only 10.11 g more per month (95%CI: -8.7 - 26.88), and infants fed with formula and breast milk gained 45g more per month (95%CI: 30.00 - 60.30). Together, these findings might suggest that 'exclusive' bottle-feeding, whether with formula or expressed milk, were the two 'riskiest' feeding methods for rapid weight gain. These findings suggest that higher proportions of bottle-feeding may result in a higher risk for RIWG as bottle feeding may promote less responsive feeding and a greater capacity for overfeeding. The authors suggest that behaviours and features associated with bottle-feeding might be modifiable intervention targets to promote healthy weight development in infancy.

In exploring this hypothesis using a unique trial design, Fewtrell and colleagues investigated whether the design of infant feeding bottles might influence infant milk intake, behaviours, or infant growth in a small RCT with exclusively formula fed infants (n=63).[55] Specifically, a 'one-way air valve' bottle (Bottle A), which would require more effort for the infant to obtain milk from, was compared to an 'internal ventilating system' (Bottle B) which would be easier for an infant to obtain milk from. Whilst the authors hypothesised that the provision of the Bottle A would result in lower milk intake and thereby slower growth, there was no significant difference in weight gain between groups (0–4-week weight gain; Bottle A = 0.74 (SD = 1.2) vs. Bottle B= 0.51 (SD =0.39). Whilst this was a small RCT, these findings did not support the influence of directly altering bottle-feeding mechanisms and designs a am effective way to shape infant growth outcomes. However, it is also of note here that the study did not compare *breastfeeding* mechanisms to bottle-feeding, and alternative behaviours or practices when feeding from the breast may contribute to different feeding experiences and carry implications for infant growth.

In the CHILD birth cohort (n=2553), Azad et al adopted a similar approach to Li et al but in an alternative Canadian cohort.[54] The findings of note are as following. Firstly, infants breastfed for longer 'duration' show more favourable weight outcomes across infancy (aβ = +0.48 BMI Zscore for breastfeeding <3 months, +0.29 for 3 -6 months, +0.19 for 6-12 months, compared with breastfeeding beyond 12 months; P for trend <.0001). Secondly, infants fed with a mixture of breastfeeding and bottle-feeding had higher BMI Z-scores than those fed only from the breast (mean: +0.14 ± 1.00 vs −0.02 ± 1.06; aβ +0.12; 95% CI: 0.01 - 0.23). However, they were still smaller than infants partially breastfed (aβ +0.28; 95% CI: 0.16 - 0.39) or not breastfed (aβ +0.45; 95% CI: 0.30 - 0.59) at 12-months. However, it remains unclear whether proportions of breast to bottle-feeding, within the 'mixed-feeding' categories may be independently associated with RIWG, and whether variations in the proportions of formula vs expressed-milk may account for variations in these effect sizes. Moreover, this study highlighted that that supplementation with formula milk appears remarkably 'riskier' for infant growth than introduction of solid foods before 6-months of age, warranting a focus on formula feeding, the circumstances under which it may lead to rapid growth, and the mechanisms which might explain these adverse growth outcomes. Finally, although Azad et al did not consider an 'exclusively expressed-fed' group as specified by Li and colleagues, so a controlled comparison between formula bottle-feeding and expressed breastmilk bottle-feeding is not possible, the mixture of formula feeding and breastfeeding led to a more marked increased in rapid growth (β =0.38, 95% CI: 0.27 - 0.48) than the mixture of expression and breastfeeding (β =0.14, 95% CI: 0.05 - 0.24). However, variations in the ratios of breast to bottle feeding may vary in these groups.

Finally, a few secondary analyses of data collected in infant feeding trials (i.e. observational studies of these trials) have highlighted associations between formula feeding and rapid weight gain. NOURISH was designed to promote positive early feeding practices (n=612)[56], and was undertaken across seven major hospitals in Australia with full term and healthy birthweight infants. The study collected detailed information on growth across infancy, as well as feeding practices, feeding modalities and demographic characteristics at 4-7 months of age. Formula feeding was associated with a higher risk of rapid growth (change in weight-for-age z-score from birth >0.67) as compared to infants fed any amount of breastmilk (OR =

1.72 (95%CI: 1.01 - 2.94), p = 0.05), adjusting for infant birthweight, introduction of solid foods, maternal BMI and age. In a more detailed dose-response analysis of IFM on rapid growth, there was no statistically significant effect (chi-squared for trend P = 0.22) although the highest proportion of infants showing rapid growth were in the formula fed group, followed by combination fed, and lastly the EBF group.

Taken together, recent studies looking at the association between IFMs, using more clear and detailed definitions of IFMs, and infant weight outcomes suggest that formula feeding is associated with promoting more RIWG. More studies from varied populations would be valuable to corroborate these results in various contexts. Furthermore, there are also several limitations which must be considered when extrapolating these results, as detailed in the following section of the literature review.

1.2.2.3 Confounding from The Social Gradient of Breastfeeding Behaviour in Observational Studies of Unrelated Infants

A persistent limitation across the observational epidemiological studies on a breastfeeding and weight outcomes is confounding from the social gradient of breastfeeding behaviour. Across high-income countries, rates of breastfeeding initiation are higher, and breastfeeding duration is longer, among women from more advantaged backgrounds.[42] In contrast, within lower-income countries, socio-economically advantaged communities show lower rates of breastfeeding in favour of formula feeding. Specific to the UK, evidence from the population-representative Millennium Cohort Study, demonstrates that mothers of lower occupational status were four times less likely to initiate breastfeeding, and mothers of lower occupational status were also less likely to be exclusively breastfeeding at both 1-month (OR 0.42, 95% CI 0.36-0.50) and 4-months (OR 0.5, 95% CI 0.31-0.77) of age as compared to mothers of higher occupational status.[43] These socioeconomic gradients are seen in numerous higher-income countries such as the US, Ireland, Germany, the Netherlands and France.[57] It is therefore plausible that in these countries, breast-fed infants may show more optimal weight patterning as result of the optimal health and development conditions provided by socio-economic advantage (such as a higher quality diet during complementary feeding and in early childhood, and different parental feeding practices), as opposed to direct effects of IFM. A recent review of the ‘breastfeeding inequality gap’, using breastfeeding surveys in 25 countries across Europe, explored the reasons for, and implications of, social grading in breastfeeding. It was observed that countries with the largest gap in breastfeeding behaviour (such as France, Ireland and the UK) had the most limited parental leave payment policies and the most widespread marketing of breast milk substitutes[57]. These findings suggest that lower rates of breastfeeding in lower socioeconomic standing groups may be driven, partly, by reduced occupational support to maintain breastfeeding and the increased psychosocial and economic pressures of limited parental leave. Hence, social positioning may not only influence a mother's opportunity and ability to breastfeed, but also a number of other social, behavioural, and health related exposures that are also strongly associated with a greater risk of rapid growth and overweight in childhood.[57]

The most recent WHO analysis of the COSI study, a comprehensive study exploring the influence of breastfeeding on child weight across 22 European countries (n=29,245), showed that breastfeeding carried a protective effect for childhood obesity outcomes when averaged across countries.[57] However, the magnitude of this protective effect varied substantially between countries. In Albania and Kazakhstan, where breastfeeding is less socially patterned, and rates of EBF are the highest (50.7% and 56.9% respectively), this protective effect of breastfeeding on later BMI did not reach statistical significance. Furthermore, in a smaller cohort of Brazilian children (n=764), where the social gradient of breastfeeding is reversed such that breastfeeding rates are *higher* amongst lower socioeconomic groups, there was no effect of breastfeeding on obesity outcomes in childhood.[58] Together, this suggests that when the influence of social positioning is removed, or even reversed, breastfeeding does not show robust benefits for childhood weight. To utilise more causal-inference methods to explore these biases further, Brion et al[59] compared the protective effects of breastfeeding on child BMI between the UK ALSPAC cohort study (n=~5000), where breastfeeding was largely socially graded, and another Brazilian Pelotas cohort (n =~1000), where breastfeeding was not related to socio-economic standing. As hypothesised, breastfeeding was associated with lower childhood BMI in UK's ALSPAC but not in Brazil's Pelotas cohort, suggesting that the link between breastfeeding and child BMI may reflect residual confounding by socioeconomic standing. However, it is also important to consider other contextual variations across countries that may lead to variations in weight outcomes. For instance, in low-to-middle income countries such as Brazil, formula feeding is associated with higher absolute risk of infection, often due to poor sanitation, which may lead to lower weight gain or weight faltering and therefore lower rates of obesity amongst more disadvantaged formula fed infants.[60] Taken together, findings from different global contexts suggest that important and systematic differences in socioeconomic characteristics between breastfeeding and formula feeding groups might account for a certain proportion of the measured benefits of breastfeeding on child weight outcomes.[61,62] However, this social patterning is rarely considered and accounted for in the breastfeeding literature. A recent systematic review by Owen and colleagues[63] highlighted that across 61 observational studies of breastfeeding in relation to obesity, a mere six (9%) adjusted their analyses for three major confounding factors: socioeconomic position, maternal smoking, and parental obesity. Moreover, when these factors were adjusted for, the association between breastfeeding and risk of obesity in later life was notably reduced, from an odds ratio of 0.86 to 0.93. Finally, Owen et al found evidence of publication bias, with the strongest protective effects of breastfeeding amongst the smallest sample sizes, as well as selective reporting of favourable odds ratios in many of the studies. In summary, observational evidence evaluating the extent to which breastfeeding may have protective effects for child and infant growth outcomes is lacking in its consideration of the social patterning of breastfeeding behaviour. Future studies and designs, which can eliminate some confounding from social positioning when testing these relationships are imperative.

1.2.2.4 Confounding from Reverse-Causation or Reciprocity in Infant Feeding Decisions

As RIWG has been established as a major risk factor for overweight and obesity in later childhood and adulthood, a prevailing worldview has emerged whereby formula feeding may be a *cause* RIWG in infancy. Hence, if fed ‘correctly’ (e.g. breastfeeding or through responsive feeding practices) by their caregivers’ children and infants can show favourable weight trajectories. However, as discussed in the current review, the association between formula feeding and RIWG has largely been observed in unique infant feeding trials[64,65] and larger observational cohorts[50,53,54]. Proposed mechanisms linking formula milk with elevated risk of RIWG include; the varied nutritional makeup of formula milk compared to breastmilk[30], a greater propensity for unintentional overfeeding when feeding through a bottle, and the reduced capacity for infants to self-regulate milk their intake when bottle fed.[66]

To date, studies of IFM and infant or child weight have focused almost entirely on the path from *parent to child* – i.e. testing the hypothesis that the way an infant is fed plays a causal role in their future weight development. However, it is also possible that there is a path from child to parent insofar as very early infant weight gain could play a causal role in parental feeding decisions – i.e. whether or not formula milk is introduced. Hence, the literature on IFMs and weight development has rarely investigated how the inner most individual child layer of the TEAM-ECD Framework (see **Figure 1.2**) might influence IFMs.

In recent years, a novel hypothesis – the reciprocity hypothesis - has emerged whereby infant feeding decisions may also be a response to the early expression of infant characteristics – such as early weight development.[40,67] For example, caregivers may *respond* to their infant's weight development by modifying their feeding practices, such as introducing formula milk to infants who are growing too slowly on their growth chart or if parents are concerned about adequate weight gain. Selection biases could influence EBF in the opposite direction as well. For example, infants may be supplemented with formula milk if they have a larger appetite and if mothers feel their breast milk supply is inadequate to nourish their fast-growing child. In this way, infants with a heartier appetite or who are growing quickly in the early weeks may be more likely to be formula fed as opposed to EBF.

The reciprocity hypothesis therefore poses that infants who are EBF are a select group of infants who did not show weight faltering in the early weeks of infancy, or who do not have an appetite which taxed their mother's milk supply. Crucially, the presence of such reverse causation in both these directions may be masked in previous observational studies, which have tended to measure RIWG from birth to 12- or 24- months[19], therefore missing the very early rate of weight gain to which parents may have responded. Future research including sufficiently repeated measures of infant weight development and early measures of infant feeding practices are needed to explore whether such reciprocity may exist. These investigations will both shed light on a more diverse set of IFM determinants than previously considered – particularly across the innermost layer of the TEAM-ECD framework. This can help shape and inform feeding interventions and guidance that consider reciprocity in early feeding dynamics between caregiver and child. Moreover, they may challenge the worldview that EBF is largely a choice which parents make in isolation of their infant's emerging characteristics. Literature exploring the reciprocity hypothesis both within infancy and within later childhood will be reviewed under the next section.

1.2.3 Reciprocity Between Infant Feeding and Infant Weight Development

Observational studies using birth cohorts or infant feeding trials, as described above, rarely have repeated measures of infant growth across the first weeks of life. These studies are therefore unable to capture bidirectional interplay in feeding and weight gain during the first few weeks of life. However, reciprocity can begin to be observed within a simple comparison of internationally used growth references in infancy, such as the WHO growth standards in the UK and the CDC growth reference data in the US. [68] The WHO growth standards are based on samples of infants who were predominantly or exclusively breastfed[11], while the CDC growth reference data were derived from a sample of predominantly formula-fed infants.[68] The WHO growth standards show a higher mean weight across the first 6 months of life compared to the CDC growth references. The higher overall weights of the predominantly or exclusively breastfed infants is at odds with the hypothesis that formula feeding leads to more rapid weight gain in infancy from birth. This suggests the higher early weight amongst breastfed infants reflects reciprocity such that they were breastfed *because* they we're growing well and had no health complications that may have led to the introduction of formula milk.

There have also been a few secondary analyses of infant growth trajectories in observational samples, which have mirrored these patterns. In a secondary analysis of the aforementioned PROBIT trial (n= 1271), infants who were given formula at some time in the first month were lighter at 1-month of age than infants who were EBF to 3 or 6 months. Moreover, these infants continued to be lighter than EBF infants until 6-months where they *caught up* with the EBF infants.[41,69] These trends are demonstrated in **Figure 1.4** below (which also shows the weight gain of the intervention and control groups in PROBIT for comparison with the observational findings). It was therefore hypothesised that a drop in weight or downward crossing of centile spaces on the growth charts may lead parents to supplement with formula milk to support their baby's growth. To explore these patterns further in the PROBIT trial, a further sensitivity analysis showed that infants who were EBF for more than 3 months or 6 months showed higher rates of weight gain until 3 months of age, and then lower rates than formula fed infants from 3-12 months of age. Hence, it appears that PROBIT infants who were EBF for 3-months (n= 1271), or 6-months (n = 251) showed slightly lower rate of weight gain from 6-months onwards, in line with the prevailing worldview that breastfeeding is protective over infant growth – although the confounding influence of complementary feeding after 6-months needs to be considered. Yet, when focusing on growth patterns prior to 6-months – where reciprocity is plausible – infants weaned onto formula milk sooner (weaned <1 month) were smaller than infants who continued to be EBF until 3- or 6-months of age. The authors therefore postulated that the introduction of formula milk might be a *response* to slower growth in early infancy.

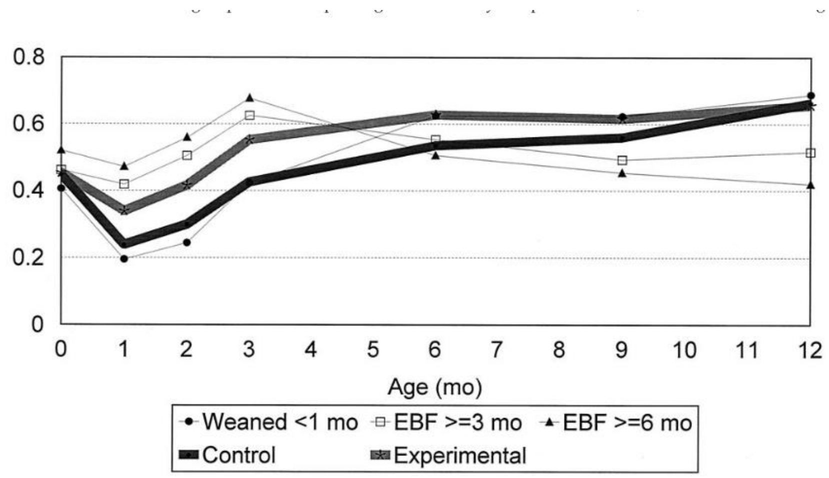


Figure 1.4. Weight for age z-score in experimental group versus control trial groups and 3 observation groups, in the PROBIT trial obtained from Kramer et al[41]

To date, only two studies have directly set out to directly explore the emerging reciprocity hypothesis in relation to early infant growth. Within a subsample of the Cambridge Baby Growth Breastfeeding Study (CGBS) (n=148), slower infant weight gain in the first weeks of infancy was associated with earlier cessation of EBF and supplementation with formula milk.[70] For infants who were EBF to 2 weeks (n=148), and infants EBF to 6-weeks (n=94), a +1 increase in weight SDS prior to formula introduction decreased the odds of EBF cessation by ~70% (OR 0.32; 95% CI 0.12 - 0.77) or ~80% (OR 0.18; 95% CI 0.05 - 0.63). In other words, slower weight gain between 0-2 and 0-6 weeks decreased the odds of parents continuing to EBF – suggesting that slower weight gain may lead to the introduction of formula milk to support infant growth. In another recent secondary analysis of the PROBIT study, applied an instrumental variable design to explore reciprocity. Here, 'compliance' to the WHO Feeding guidelines within the breastfeeding promotion intervention group was used as an instrument to represent parental response to steadier infant growth and low parental concern about growth.[40] Infants of compliant mothers (those who continued to breastfeed with any breastmilk for ≥12 months showed steadily higher weight in the first two months of infancy. Conversely, infants of non-compliant mothers (breastfed for <12 months) showed a drop in weight z-score between birth to 2-months of age. This finding suggests that longer duration of breastfeeding may be driven by steadier and faster weight gain amongst infants in the early weeks of life, supporting the reciprocity hypothesis. However, it is also of note that infants of noncompliant mothers demonstrated an increase in their rate of weight gain after 2-months – slightly exceeding the final weight of infants whose parents were compliant. Hence, this study also indicates that the introduction of formula milk may be an effective strategy to increase infant weight gain or 'catch-up' in growth, for infants who grow slowly in the early infancy period. Whilst this study is one of the few to explore longitudinal weight gain trajectories in the context of the reciprocity hypothesis, the indicator of 'compliance' represents a high duration of Breastfeeding (≥12 months), not EBF. Therefore, both groups of infants received both formula milk and breastmilk – in varied combinations. This may dilute the effect of reciprocity – as mothers may be 'compliant' but offer substantial formula milk whilst continuing to breastfeed. This measure may have also diluted the influence of 'non-compliance' or formula feeding on later growth – as mothers may have been 'non-compliant' (breastfeeding <12-months) but breastfed for a considerable duration, which was common in PROBIT.[41] Hence, it would be of value for future studies with more specific measures of IFMs and repeated measures of infant growth to explore the reciprocity hypothesis.

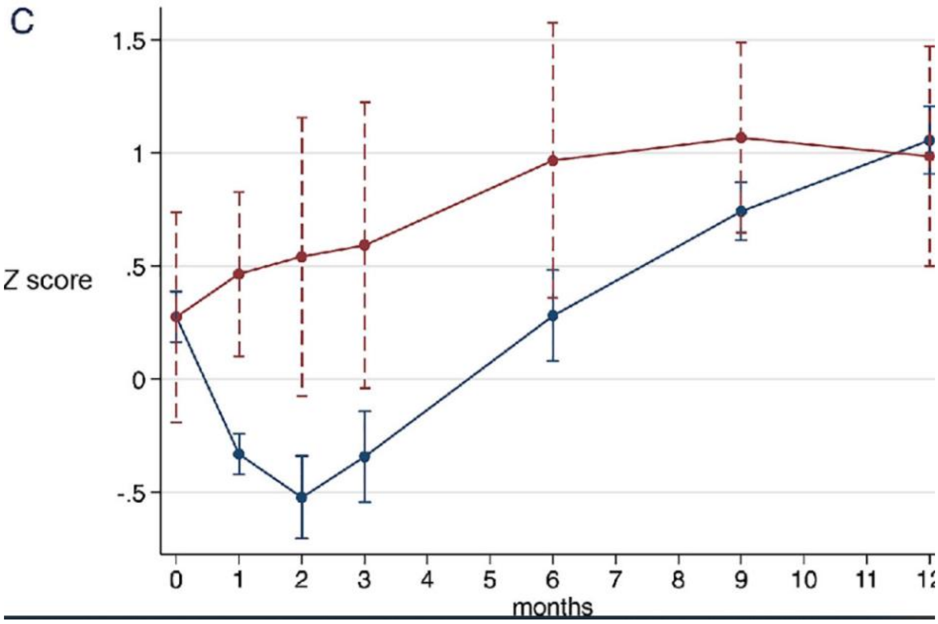


Figure 1.5. Weight for age z-score trajectories in Instrumental Variable Analysis of PROBIT; comparing infants who were breastfed (with any breastmilk) for ≥12 months (red) with those who were breastfed (with any breastmilk) for <12 months (blue) obtained from Kramer et al[41]

Finally, the reciprocity hypothesis can also be supported by more qualitative insights from the UK Infant Feeding Survey (2010) the most recent national survey capturing how caregivers in England feed their infants in the first year of life. In which, most of the mothers who were formula feeding reported a desire to EBF for longer, yet one of the most common reasons for EBF cessation was concern for infant weight.[32] Together, these observations support the hypothesis that parents respond to the growth of infants in their feeding decisions. However, more studies containing sufficient measures of infant growth – prior to and after – the introduction of formula milk are needed to test the presence and strength of these bi-directional relationships in infancy. Moreover, these insights also suggest that it would be of value to consider whether both actual weight gain and perceptions or concern for infant weight gain might influence infant feeding decisions.

1.2.4 Reciprocity Between Parental Feeding Practices and Child Characteristics

Whilst studies that test the reciprocity hypothesis in infancy are scarce – it has been demonstrated that both parental feeding practices (PFPs) in later infancy and the introduction of solid foods may be responsive to weight development.[71,72] In one observational study of PFPs (n=1950) in the Gemini twin cohort, more pressured feeding was performed in response to a lower birth weight, suggesting that parents modify their milk feeding behaviours in response to anthropometric characteristics of their children.[73] In regard to the introduction of solid foods, a recent review of studies (n=15) demonstrates that RIWG tends to precede the early introduction of solids. Hence, choosing to supplement milk with solid foods may be a *response* to a child growing more quickly – potentially to keep up with their growth or larger appetite.[74]

However, most of the literature considering reciprocity between parental feeding practices (PFPs) and weight development have focused on childhood. Similar to infant feeding practices, the vast majority of the literature examining relations between PFPs and weight are cross-sectional, presenting associations between restrictive feeding (e.g. restricting food intake), pressured feeding (e.g. pressuring child to eat more or finish everything on the plate), indulgent feeding (e.g. setting few limits around food intake) and risk of overweight or obesity across childhood[75–77]. Yet, a few studies have looked at relationships in the opposite direction – from child to parent. In one American birth cohort, increasing BMI between 4 and 7 years of age was associated with more controlling parental feeding practices (PFPs) from 7 to 9 years of age (n=789).[72] No association was found when looking at the pathway from controlling PFP to BMI, suggesting that PFPs become more controlling following increases in weight rather than before. Similarly, in the Generation XXI birth cohort (n=708), higher BMI at 4 was associated with higher restriction and control at 7 years of age, and this path from child to parent was stronger than the opposite direction. [78] In light of these findings, it has been speculated that parental concern and perceptions of weight may *mediate* or explain relations between weight gain and PFPs. Hence, PFPs may be responses to concerns about their child's weight development. In this way, a mother's concern for their child's excessive or low weight gain may be the driver of more controlling and restrictive PFPs. In support of this hypothesis, Webber and colleagues found that maternal concern for child overweight fully mediated a cross-sectional association between higher child BMI and higher restriction (n=405).[79] Similarly, in a larger Dutch cohort (n=4689) cross-lagged models demonstrated that higher BMIz at age 4 predicted more restrictive PFPs at age 12, and maternal concern about child weight partially mediated these relationships. [80] Another study demonstrated reciprocity in PFPs as early as pre-school years, with a higher BMIz at age 2 associated with higher restriction at age 4 (n=4166), but they were not able to explore mediation by concern.[71] Whilst these studies contribute to an emerging evidence base for parent-child reciprocity, residual confounding from other unmeasured (or poorly measured) factors may also explain the observed associations. Hence, more rigorous designs such as co-twin designs, which are able to remove residual confounding from shared environmental factors, would be useful to shed light on this relationship in a 'real world' context.

An emerging evidence base for reciprocity in child feeding in relation to a child's unique appetite traits is also emerging. I recently co-authored a study in Gemini to explore observational reciprocal associations between PFPs and child eating behaviour within early childhood.[81] In this study, greater emotional overeating in the child was predictive of greater instrumental feeding ($\beta = .09$; 0.03–0.15; $p = .005$), and a child's greater enjoyment of food predicted greater encouragement to eat nutritious foods from their parents ($\beta = .07$; 0.02–0.11; $p = .003$). Twins also offer a powerful design to testing the hypothesis that parental feeding decisions are developed partly in response to their child's emerging characteristics. This is because twins share their environments to a very similar extent (e.g. brought up in the same home, by the same parents, and are exposed to the same social environment in early life), so if parents feed them differently, the most plausible explanation is that this is a parental response to individual differences in the behaviours or experiences of the two twins. Designs such as the Discordant Twin (DT) design can identify individual differences between twins that shape parental feeding decisions across childhood. Whilst no DT designs have been applied to explore reasons for discordance in infant feeding, few studies explore whether PFPs in later childhood were responsive to child appetite. Whilst this review is centred on reciprocity to infant and child weight development, not appetite, this example can help illustrate how twin designs are ideal to highlight potential reciprocity in the parent-child feeding dynamic. I also co-authored the most comprehensive co-twin study to date on reciprocity between child appetite and PFPs, in toddlerhood and early childhood.[82] Within this study, parents used more pressured feeding with a co-twin with lower parent-reported food responsiveness, lower emotional overeating, lower food enjoyment, as well as; higher satiety responsiveness, slower speed of eating higher emotional undereating and greater fussiness (n=122 – 544 twin pars). Moreover, Harris et al. demonstrated that mothers used more pressuring (e.g. pressuring a child to finish a meal) and instrumental (e.g. being more rigid in feeding schedules) PFPs in toddlerhood with their twin who expressed more fussy eating profile than their co-twin.[83] Together, these twin studies also contribute to the emerging evidence base highlighting PFPs as responsive to children's traits from early childhood.

Together, these novel but rare twin studies suggest that reciprocity in the parent-child feeding dynamic and must be explored more widely. To date, few twin, or even sibling, studies have been utilised to unpack reciprocity within infancy - a clear gap in the literature.

1.2.5 Summary of Infant Feeding Methods and Weight Development

Taken together, the literature exploring relationships between IFMs and child weight development has largely focused on the *parent to child* pathway, exploring whether IFMs influence child weight development using observational methodologies. On the whole, observational studies of unrelated infants indicated that breastfeeding (whether exclusively, predominantly, or for longer) is associated with a lower risk of RIWG and more favourable weight development in later childhood. However, these observational studies have numerous limitations which need be addressed by future studies; mainly in regard to the crude measures of breastfeeding behaviours, limited repeated measures of infant weight, and limited ability to remove residual confounding from the social environment. Moreover, studies which have been able to use more rigorous experimental (i.e. infant feeding trials) and quasi-experimental (i.e. twin studies) designs have not consistently supported the relationship between IFMs and infant weight development. One hypothesis, the reciprocity hypothesis, has therefore emerged whereby these results might reflect reverse-causation from reciprocity in the infant-caregiver feeding relationship. In other words, early growth patterns might influence infant feeding choices themselves. As literature from later children, shows convincing evidence of bi-directionality and few studies in infancy have supported the reciprocity hypothesis – reciprocity must be explored in relation to IFMs urgently.

Addressing this gap by exploring the extent to which infant feeding decisions might be a response to infant weight development using the co-twin design has numerous implications. Firstly, it will help to disentangle the complex web of factors involved in shaping infant feeding decisions – such as the introduction of formula milk – and therefore identify key targets for infant feeding interventions. Secondly, evidence of reciprocity can help to disrupt the prevailing view that parents are 'to blame' for their child's early weight development, encouraging a more nuanced understanding of how child characteristics and feeding practices interact in infancy. Finally, these findings may support the utility of more personalised and responsive infant feeding guidance – whereby parents can be offered feeding support that is tailored to their experiences of feeding their child and therefore more relevant to supporting the healthy development of their child.

1.3 Gene-Environment Interplay in Infant Feeding Methods and Weight Development

The thesis aims to explore interactions between the layers of the TEAM-ECD model and their influence overweight development in the first year of life. The previous section highlighted findings regarding the influence of IFMs on infant weight development (at the individual family layer), and then went on to focus on the scarce but emerging literature exploring reciprocity between IFMs and infant weight development (an individual and family layer interaction). This section will also consider interactions between the individual and family layer – but focus on gene-environment interplay. This review will highlight key gaps amongst these studies – such as unexplored topics and methodological limitations, which can be addressed to better understand how infant feeding practices and genetic influences interact to shape weight development across infancy.

1.3.1 Heritability of Infant Weight Development

Heritability is defined as the degree of variation in a phenotypic trait, such as weight gain, which can be attributed to genetic variation within a population.[84] Put simply, heritability is a measure of the extent to which differences in people's genetic liabilities account for differences in their traits (it ranges from 0-100%). However, several caveats when interpreting Heritability are worth reflecting on as they can be often misunderstood. First, heritability is not the proportion of a trait that is genetic, but the proportion of *variance* in a trait that is influenced by genetic factors. Second, heritability is measured as a population parameter – and therefore it does not predict the value of heritability in other populations. Finally, it is important to appreciate that heritability for a single trait is not constant across the life course. Heritability estimates for a trait can change over time as; the variation applicable to environmental factors can change, or the interaction between genes and the environment (GE interplay) change. It is important to consider how heritability of weight development across the life course may vary in a complex manner.

Decades of twin studies have established substantial heritability for Body Mass Index (BMI) from infancy to adulthood, with estimates ranging from 50 to 90% (N=84,782) from 1 to 18 years of age.[85] Recent advances in genotyping have also highlighted the polygenic nature of childhood BMI, implicating thousands of genetic loci in the development of body mass from early childhood onwards.[86] Whereas monogenic traits are a product of a single gene, polygenic traits are the result of many genes. Polygenic susceptibility has also shown associations with infant weight at 1-year of age, supporting the early emergence of genetic influences on weight development.[87] When twin studies have been used to estimate heritability in the infancy period, heritability estimates vary across the first year of life.[88–90] In the Gemini Twin Study (n= 2402 pairs), from birth to three months of age, variation in growth is more attributable to environmental influences (i.e. infant feeding exposures) than genetic factors as heritability was low (38%).[91] However, by 6-months infant growth is largely attributable to genetic factors (66-90%), with high heritability estimates for both infant size (weight) and velocity (speed of growth). These findings have been replicated in a few infant twin cohorts. These include the East Flanders Prospective Twin Survey (n=2011 pairs) where genetic influences on weight z-scores were significant at ages 12- to 24-months but not 0 to 1 months.[88,92] The Fels Longitudinal Study (N=501; USA) also demonstrated significant heritability estimates for infant weight status ($h2=0.61$-0.95) at every time point from birth to 24-months of age, as well as change in weight z-score ($h2=0.56$-0.82) from birth to two years.[93] The Netherlands Twin Register (NTR) cohort (n=2649 twin pairs) showed higher genetic influences on weight (kg) and weight velocity at 1 and 2 years of age, than on birthweight.[21] For instance, the variation in weight velocity parameter was largely explained by additive genetic factors (57% to 63%), whilst variation in birthweight was less so (14 to 24%). Finally, a twin analysis of the larger combined Dutch Generation R Study and Netherlands twin register (N= 33,694 twin pairs) mirrored these results, with heritability estimates for weight z-scores increasing from 29% at birth to 71% at 36-months of age.[94] Together these findings suggest that in very early infancy, variation weight may be under stronger influence from shared environmental (e.g. social positioning) and non-shared environmental (e.g. infant feeding exposures) factors, yet by 6-months genetic influences become a more important influence over weight development. Although this varies across cohorts. However, as these twin studies have been undertaken in western and developed countries, it is important to recognise how they may not extend to other populations with differing food-environments.

Moreover, crucial to the concept of heritability is also a consideration of how genetic influences might interact with a child's environmental exposures to influence weight development. Significant variation in heritability has been observed (31 – 90%) in heritability estimates of BMI in later childhood – with higher heritability in populations living in more deprivation, higher average BMI, as well as in cohort studies born more recently.[95] In one analysis spanning numerous genetically sensitive birth cohorts – the association between a polygenic risk score (PRS) for obesity and adult BMI was more pronounced in younger cohorts (i.e. those with a more recent year of birth).[96] One explanation for this may be that younger cohorts have spent a greater proportion of their lifespan exposed to an obesogenic environment, which allows for greater expression of genetic risk. This might suggest that adiposity-promoting genes are expressed to a greater extent in more obesogenic environments – an example gene-environment (GE) interplay. Although the genetic basis of early weight development is well demonstrated, it is less clear how genetic susceptibility towards early growth patterns interacts with the environment in such a way. In line with this hypothesis, it is important to consider how the twin design models' gene-environment interactions and correlations as additive genetic effects (A) using the traditional univariate twin model. This is because MZ twins are not only more genetically similar than DZ twins, as they share 100% of their genetic material versus ~50%, but also because MZ twins might also react to environmental exposures more similarly than DZ twins.[97] Hence, as the classic twin model inherently assumes greater correlation between MZ twins in a phenotypic trait is due to greater genetic similarity, but MZ twins might also select into more similar environments or respond to environmental exposures more similarly due to their co-twin relationship. Hence, additive genetic effects might capture some important GE interplay. For a wider review of this phenomenon and how it can be addressed under careful twin comparisons see Verhulst et al.[97] In short, twin methodologies that go beyond the classical univariate model can be useful to disentangle potential GE interplay – as described further in Chapter Two Section 2.5.5 of this thesis.

1.3.2 What are GE Interactions?

In recent years, there has been increasing interest in GE interplay. It has been postulated that GE interplay may account for demonstrated variations in the heritability estimates of child weight across varying populations and environments.[98–101] For instance, children exposed to more obesogenic environments (e.g. higher exposure to higher-caloric foods and fewer opportunities for physical activity) may encounter more opportunities to express their genetic risk towards adiposity – leading to higher heritability over variance in weight development. However, when discussing GE interplay it is critical to distinguish between the different forms. In a landmark paper, Plomin[102] distinguished between GE correlation and GE interactions – which are often used interchangeably yet represent different ways in which our genetics and environment interact. GE correlation represents the extent to which our genes can influence our exposures to certain environments or environmental exposures. Three forms of GE correlation have been investigated. **Active GE correlation** is when children actively select environments which are correlated with their genotype (e.g. choosing an after-school club that reflects their genetically influenced characteristics, such as a passion for music). **Passive GE correlation** is when children passively inherit environmental conditions related to their genetic liability (e.g. parents who enjoy reading provide a home full of books for their children, who also, by virtue of their genes, enjoy reading). Finally**, Evocative GE correlation** occurs when children with a certain genetic liability evoke a particular reaction from their environment or carers which go on to influence their outcomes (e.g. infants who have a genetic predisposition to faster weight gain may be fed differently than infants with a genetic predisposition to slower weight gain).

Across the past few decades, numerous models and theories have emerged to help better explain the nature of gene-environment interactions.

One particularly relevant model, the behavioural susceptibility theory[103], explains how the modern obesogenic environment interacts with children's genetic liabilities, such that it does not affect all children equally. Genetic influences on appetite, which are present from birth, modify children's response to obesogenic environment. A child with a genetically influenced larger appetite may be more vulnerable to the food environment through an active GE correlation, whereby they select into environmental exposures and feeding practices correlated with their genetic liability. Moreover, evocative GE correlation may present itself from the start of life, where infants' genetic liability for early weight patterning, or appetite traits, may explain why parents modify their feeding practices in response to their child.

On the other hand, **GE interactions** represent the extent to which the expression of our genetic liabilities can be influenced by certain environments or exposures. A GE interaction relevant to the current thesis would represent a scenario where infants who are fed with more obesogenic or adiposity promoting feeding practices are given the opportunity to *express* their genetic liability towards higher weight gain. A better understanding of both GE correlation and GE interactions is important for a number of reasons. First, GE interactions can help to better pinpoint environmental risk factors, such as protective feeding exposures, which could buffer a child's expression of genetic risk and therefore support healthy weight patterning for those at greater inherent risk. Whereas an understanding of GE correlation can highlight ways in which parents may adapt to their children's genetic liabilities – therefore suggesting that those of higher liability may be particularly benefit from intervention and support that targets risky feeding practices. In this way – genes are not destiny when it comes to infant weight development, as is hardly ever the case with complex phenotypes. Instead, genetic liability might *influence* or *be modified by* early feeding practices and therefore be important to understand alongside environmental risk factors. In sum, a theoretical understanding of infant feeding practices and infant weight development without the consideration of genetic susceptibility and GE interplay would be incomplete.

Few GE interplay studies to date have considered the infancy period, given the challenge of collecting genetic data from samples with sufficient phenotypic measures – such as infant feeding practices and weight gain. Nonetheless, systematic reviews of twin studies have shown significant heritability – around 23% - across various measures of parenting behaviours.[104] Measuring genetic influences on parenting behaviours is one way to measure how parents might be responding to their child's genetic liabilities. High heritability estimates for variation in feeding practices – would suggest that parents are modifying their feeding in response to the genetic liability of their child – an evocative GE correlation. Crucially, such findings can challenge the commonly presumed top-down influence of parental feeding behaviours on child weight development. Whereas in reality parental behaviours may in fact be influenced by their child's genetic susceptibilities.

Similarly, in relation to GE interaction, the extent to which genetic susceptibility influences a child's weight could also be moderated by the environment they grow up in. For instance, two children with the same genetic risk for BMI could show different weight outcomes at 5 years of age if one child had been exposed to a more obesogenic environment or a poorer quality diet than the other child. This is because exposure to obesogenic environments may give a child more opportunities to *express* their genetic liability for higher weight. Although such GE interactions have been hypothesised to exist in regards to infant feeding exposures[105], few studies have measured their presence. At present, genetically sensitive approaches that are able to explore GE interplay include: i) twin studies that rely on comparisons between MZ and DZ twins[35] (see section 2.5 for a description of these methods); or ii) genomic studies, which explore whether genetic variants in obesity related genes[106], using either single or multiple variants combined into polygenic risk scores (PRS), show varying associations with weight outcomes dependent upon environmental factors. Whilst both carry merits and limitations, twin studies provide greater power to detect GE interactions than genomic studies, and crucially they are not limited to the existence of robust PRS which explain a substantial proportion of variation in a trait of interest (e.g. RIWG in the case of this thesis). At present there is no available PRS specific to weight gain in infancy – limiting the plausible GE studies that can be undertaken on infant weight gain itself using polygenic approaches. Moreover, to date, majority of twin studies exploring GE interplay have done so in childhood rather than infancy. Hence the following review will present the scarce but relevant literature regarding both GE interactions and GE correlations in both infancy and childhood.

1.3.3 GE Interplay in Infant Weight Development

Infant weight patterns - such as weight and weight gain trajectories during the first year of life - appear to be under genetic influence as highlighted in the previous section. Although genetic influence on weight emerges in infancy, this does not therefore mean that early weight outcomes, such as RIWG, are *destined* or *unmodifiable*. Environmental factors shared entirely by twin pairs explained 21% of variation in change in weight z-score from birth to 6-months of age in the Gemini twin cohort, whilst environmental factors unique to each individual twin explained 22% of variation in change in weight z-score.[91] Moreover, environmental influences may also be crucial to understanding RIWG through its interplay with genetic risk. Whilst both genes and the environment have direct influence over RIWG – they also interact to shape infant weight trajectories. With regard to GE correlation, infants at higher genetic risk for RIWG may also experience higher risk feeding practices if their parents respond to their genetically influenced behaviours by feeding them more. This might mean that children inherently at greater risk of RIWG may be exposed to riskier feeding practices which continues to contribute to more rapid weight gain. This may result in a 'vicious' cycle whereby genetic risk towards RIWG might be exacerbated if not considered in the child-parent feeding dynamic. Hence, it is crucial to better understand both; i) how the use of certain infant feeding practices might enable a greater expression of genetic liability for infant weight gain (a GE interaction), ii) how genetic liability for infant weight gain might influence caregivers use of certain infant feeding practices (a GE correlation).

GE Interaction in Infant Weight Development

As formula fed babies have been shown to exhibit more bottle-emptying than breastfed babies in later infancy[107] and observational studies have demonstrated the common occurrence of parents feeding infants above the WHO recommended guidelines for formula milk[24] – it is conceivable that bottle feeding, as compared to breastfeeding, may enable infants to express their genetic susceptibility towards adiposity in early life. This is because formula-fed infants are fed through a bottle, which allows for more rapid consumption of milk and therefore it has been speculated that this may increase the potential for a caregiver to override the infant's own internal satiety mechanisms, disrupting appetite regulation.[50,108] It is also possible that the different nutritional makeup of formula milk[109], might promote faster weight gain or hindered appetite regulation. Regardless of these potential mechanisms, being fed with formula in infancy may provide an infant with more opportunity to express their genetic liability for infant weight development – **an example of GE interaction**.

One genomic study to date has explored this hypothesis. Within the UK-based Avon Longitudinal Study of Parents and Children (ALSPAC) cohort, Wu and colleagues reported that EBF, versus formula feeding, could amplify the association between a genetic risk score for BMI (97-SNP Polygenic Risk Score (PRS)) and BMI trajectories from infancy to 20 years of age (n= 5,266).[110] EBF to 5-months had a stronger protective effect for children with a higher PRS score for BMI. This protective effect also became more apparent with age in both boys and girls. For example, in girls, EBF to 5-months decreased BMI at 7-years by 0.62 km/m^2 in the highest PRS risk group (95% CI, 0.28 - 0.96, p = 0.0003) versus 0.38 kg/m2 (95% CI, 0.04 - 0.72, p = 0.0272) in the lower PRS group. At 18 years, these differences were larger with a decrease of 0.86 kg/m^2 (95% CI, 0.11 - 1.62, p = 0.0252) in the lower PRS group as compared to 1.53 kg/m^2 (95% CI, 0.76 - 2.29, p<0.0001) in the highest PRS risk group. As such, EBF to 5-months of age moderated the relationship between the PRS and actual BMI at 18-years of age in both boys (reduction of 1.14 kg/m^2, 95% CI, 0.37 - 1.91, p = 0.0037) and girls (reduction of 1.53 kg/m^2, 95% CI, 0.76 - 2.29, p<0.0001).[110] However, there was a less marked protective effect of non-exclusive BF to 5-months of age on weight trajectories. For example, in girls – non-exclusive breastfeeding decreased BMI at 18-years by 0.31 kg/m2 (95% CI, 0.04 to 0.72, p = 0.0272) in the lower PRS group as compared to 0.37 km/m2 in the highest PRS risk group (95% CI, 0.28 to 0.96, p = 0.0003); a small difference at the population level. On one hand, this finding could be inferred to reflect the need for EBF for this GE interaction or buffering effect to persist. Or, perhaps, it might also reflect social confounding, such that EBF infants in ASPAC are demographically different to non-exclusive breast feeders, and these demographic differences are responsible for the difference in later BMI as the benefits of socioeconomic advantage accumulate.

Nonetheless, the authors were also interested in how feeding exposures might modify genetic expression over weight within the infancy period with regard to the timing of adiposity peak (AP) and adiposity rebound (AR), with earlier AP and AR both associated with future obesity and cardiometabolic risk.[111] EBF was found to delay the age of adiposity peak (AP) – a protective growth pattern – more so in boys with either average or high level of PRS risk as compared to lower PRS risk. Whilst for Adiposity Rebound (AR), EBF delayed the timing of AR in girls at all levels of the PRS equally, countering any indication of GE interaction. Together, the authors suggest that GE interactions on weight development may be less prominent in infancy as compared to later adolescence. However, it is also of note that this study measured AP and AR, as opposed to more clearly established risk factors for adiposity such as RIWG or infant growth trajectories. Moreover, the risk of social confounding remains. Children who are EBF to 5-months may experience a very different environment, such as healthier diets or less obesogenic feeding exposures, given the social gradient of breastfeeding behaviour in the UK.[112] Therefore, EBF may represent a marker for social advantage which leads to starker GE interactions in adolescence versus infancy, as the protective influences of social advantage accumulate across childhood. Finally, genetic confounding may be present within IFM choices themselves (a GE Correlation), which was not explored in this study. For instance, children with a genetic predisposition to higher BMI may be fed in certain ways that promote excess weight gain, as a response to their early growth trajectories. However, the authors did not present variation in PRS scores between the EBF and or formula feeding infants, which leaves an important question unanswered. Is EBF itself genetically influenced? Future studies are needed to answer this question, measuring both GE correlation and GE interactions between IFMs and infant weight development.

GE Correlation in Infant Weight Development

To date, no published studies have explored GE correlation between IFMs and infant weight using either twin designs or molecular genetic methods. Nonetheless, the presence of GE correlation can, in part, be theorised by recent findings from the UK-based CBGS study (N=148). In this study, the introduction of formula milk was responsive to slower early infant weight gain.[70] The wider literature on parent-child reciprocity, summarized in section 1.2.3, also points towards feeding practices as early as infancy representing a response to weight gain. In summary, infants who are growing slower or gaining less weight appear to be more likely to be switched to or topped up with formula milk – a potential **GE correlation**. However, GE correlation can only be inferred from these studies on reciprocity, as parent responses have been measured in relation to actual infant weight outcomes, rather than genetic influences over infant weight outcomes. Hence, there is a need to explore whether parents modify their infant feeding practices in response to their child's genetically influenced weight development. Such research would be the first to demonstrate parent-child reciprocity in relation to genetic influences over weight from the very start of life.

1.3.4 GE Interplay in Child Weight Development

Whilst very few studies have explored Gene-Environment interplay within infancy, a few genetically sensitive investigations have measured GE interplay in toddlerhood and later childhood.

The TEDS twin cohort in the UK reported GE correlation between parental feeding practices and child weight, insofar as parents whose child had a higher polygenic risk score (PRS) for BMI used more restrictive feeding practices at 10 years (n=10,346). At the same time, parents whose child had a lower PRS for BMI pressured their child to eat more.[113] This study applied the twin design to show that co-twin differences in PRS was associated with differences in restrictive and pressured feeding, removing residual confounding from shared environmental and genetic influences. Finally, using a multivariate genetic analysis, restriction (A=43%) and pressure (A=54%) were both moderately to highly heritable, and there was a genetic correlation between higher child BMI and higher parental restriction (rA = 0.28), and lower child BMI and higher parental pressure (rA = -0.48). This suggests an important overlap in the genetic influences over parental feeding behaviour and children's BMI, suggesting that parental feeding behaviour may be influenced by genetic influences over child BMI. Taken together, this study triangulated both genomic data and the twin design to demonstrate that parents modify their feeding practices in response to their child's genetic predisposition to higher or lower weight. Authors suggested that parents were restricting children with a higher PRS score for BMI because they had a more demanding appetite or were gaining weight faster than desired – although they did not test this directly. The moderating role of appetite has explicitly explored within further studies – we however have seen more mixed results. For instance, in the RESONANCE cohort, Jansen and colleagues (n=197)[114] found that the positive relationship between a child's BMI PRS and restrictive feeding was moderated by child's food responsiveness. Parents in this case used more restrictive feeding practices in response to their child's higher genetic risk for BMI when the child showed a more avid appetite and interest in food, as compared to a less avid appetite. This aligned with Selzam and colleagues' interpretation of their results. Moreover, this study also highlighted that teaching about nutrition was negatively correlated with a PRS for BMI risk – such that children at higher risk are less likely to be taught about nutrition. Again, perhaps indicative of GE interplay. However, a larger French cohort (n=932) of toddlers did not demonstrate any association between parental feeding practices and children's polygenic risk score for BMI when measured.[115] Jansen and colleagues postulated these contradictory findings may have been due to differences in the age at child the feeding practices were measured or the difference PRS calculation methods. Moreover, perhaps feeding practices alternative to those measured by the CFPQ[116] tool - such as *what* parents are offering – may be more responsive to their child's genetic risk and genetically expressed appetite. Yet these GE correlations have not been explored to date. Nonetheless, interventions seeking to improve parental feeding practices and reduce childhood obesity may benefit from acknowledging that parents can respond to their child's genetically influenced behaviours. In one recent exploration of GE interplay using innovative causal inference methodologies, Herle and colleagues used ALSPAC to explore whether a hypothetical intervention that changes the distribution of parental feeding practices at ~10 years of age could mitigate genetic liability towards a higher BMI (n=4248).[117] In other words, how much difference in childhood BMI would remain between children of a higher and lower genetic risk, should parental feeding practices be modified through a successful hypothetical intervention. Firstly, this study corroborated Selzam et al's measured association between more restrictive feeding amongst children of higher genetic liability towards BMI. Secondly, the result of this main *hypothetical* intervention, which would shift parental feeding behaviours for children of higher genetic liability to what they would have been for the children of lower genetic liability, resulted in smaller differences between BMI outcomes between the children of higher and lower genetic risk. In other words, interventions which improve PFPs could help buffer the expression of genetic risk towards a higher BMI in those most genetically vulnerable. Importantly, this could reduce weight inequalities between children of higher and lower genetic liability by targeting important GE interplay. Moreover, interventions might also be particularly useful when targeted towards children and families at greater genetic risk. However, more studies with varying populations, more measures of feeding behaviours and child weight, and reliable genetic measures are needed to corroborate the presence of GE correlations in childhood as well as infancy to build the evidence base for such interventions.

Only a few studies to date have explored **GE interactions** in childhood using genomic measures of genetic liability. For instance, some studies have shown that the FTO gene – involved in the development of adiposity from childhood – is more strongly correlated with BMI amongst children living in lower versus higher socioeconomic settings (n=4406).[118] However, FTO is only a single genetic marker of obesity, hence there is a need to explore gene-environment interplay using more designs that capture more genetic liability such as the twin design. In regard to twin methodologies, within Gemini Twin study the heritability of BMI at 4-years of age was nearly twice as high for children living in more obesogenic home environments (86%) as compared to children in less obesogenic home environments (39%). This suggests a modification of genetic expression on weight depending on the family obesogenic environment.[119] Children living in more obesogenic home environments may therefore have more opportunities to express their genetic risk which leads them to a higher BMI – a GE interaction. Moreover, Horn and colleagues found that heritability estimates for BMI (n=5,079 twin pairs) were higher in neighbourhoods of lower walkability.[120] These findings suggest that in addition to micro-level markers of the obesogenic environment, such as the home food environment, macro-level markers at the community level may also influence the expression of obesity-related genes. Nonetheless, it is hard to control for residual confounding from interrelated aspects of the home and social environment in such designs.[120] For instance, families living in more walkable neighbourhoods might also have additional access to healthier foods, less air pollution, and less exposure to food-marketing which might each provide opportunities for the buffering of genetic risk. Moreover, it important to consider that whilst both these studies concluded that GE interaction may be present in regard to the home obesogenic environment and social positioning, it is possible that the findings can also be explained by GE correlation. For instance, certain environmental exposures encapsulated within the obesogenic home environment – such as feeding practices – may be responsive to a child's genotype. Hence, where possible, models that consider both GE correlation and GE interaction are needed. Hence, this thesis aims to explore both aspects of GE interplay. Moreover, given the focus on infant weight development in the current thesis and the lack of any GE interaction studies in infancy – I will focus on interactions between genetic liability and the environment at the proximal family layer influence by examining IFMs.

1.3.5 Summary of Gene-Environment Interplay

In summary, few studies have sought to explore potentially important GE interplay in the infancy period using genetically sensitive designs. Where GE interplay has been explored in later childhood, few studies have explicitly sought to disentangle GE correlation from GE interactions. The presence of either GE correlation or GE interactions may have important implications for infant feeding interventions and infant weight development. Firstly, if GE interactions are detected, early environmental feeding exposures – which are modifiable through intervention and policy efforts - have the potential to *buffer* the expression of genetic risk towards adiposity from the very start of life. Hence, these environmental exposures are worthy of intervention through their direct and indirect influences on weight through GE interplay. Secondly, if GE correlations are highlighted, the presence of parent-child reciprocity in feeding – where caregivers respond to their child's early expressed genetic risk – is crucial to consider. Interventions and guidelines may be more effective if they consider reciprocity and/or are tailored to challenges that emerge from a child's genetic liability for weight gain. Parents of infants with greater susceptibility towards higher adiposity might also benefit from additional feeding support, intervention, and guidance given the presence of GE interplay. Finally, they have the potential to shift the 'blame' attached to the commonplace view that feeding has a top-down and one-directional influence on infant growth.

1.4 Summary of Literature Review for Part One of Thesis

The present literature review highlights numerous opportunities to further understand and disentangle bio-psycho-social interactions in infant feeding and infant weight development. First, observational studies largely indicate that breastfeeding (whether exclusively, predominantly, or for longer) is associated with a lower risk of RIWG and more favourable weight development in later childhood. However, these observational results are limited such that they i) often use crude measures of breastfeeding, particularly in families using 'mixed-feeding', ii) use cross-sectional measures of infant weight gain and iii) are limited in their ability to remove confounding from the social gradient of breastfeeding behaviours - such that benefits of breastfeeding may represent a proxy for benefits of social advantage. Second, the field of infant feeding has largely focused on the direct *parent to child* pathway, exploring whether infant feeding methods influence child weight development using observational methodologies. Nonetheless, there is a growing evidence base supporting the emerging reciprocity hypothesis – such that child feeding is responsive to child characteristics such as weight development. As few studies have considered or tested for reciprocity in infant feeding, there is an urgent need to explore whether infant feeding practices are made in response to an infant weight development. Quasi-experimental and the twin design offers powerful tools to explore such parent-child reciprocity. Third, few studies to date have explored potential gene-environment interplay in relation to IFMs. However, emerging evidence from twin studies in later childhood indicate that PFPs are, in part, performed in response to children's genetic liability for weight development (a GE Correlation) and that expression of genetic liability overweight might be moderated by a child's home environment and feeding exposures (a GE Interaction). Future studies need to apply the twin design to better understand how genes and the environment interact to shape infant weight gain – representing an important bio-psycho-social interaction.

1.5 Overall Aims of Thesis

The overall aim of this PhD is to explore the bio-psycho-social interactions in the relationship between infant feeding and infant weight development, exploring reciprocity between IFMs and infant weight gain (Part One of Thesis - Chapters 3, 4, and 5). Specifically, my research will be undertaken on the UK Gemini sample which is a population from a high-income nation. Hence, causal pathways and relationships between infant feeding on infant growth in lower-income and less developed populations may differ from those presented in this thesis. In the second part of my thesis, I translated these insights to the development of BRIGHT - a digital intervention seeking to reduce RIWG among formula fed infants by supporting responsive bottle-feeding (Part Two of Thesis – Chapter 6). The literature review for part two of this thesis is presented in Chapter Six. The aims and the key research questions relevant to each chapter are summarised here.

Study 1; Infant Feeding Modalities and Infant Weight Gain Trajectories Across the First Year of Life, sought to explore whether a range of infant feeding modalities are associated with weight gain trajectories across the first year of life in the Gemini Study – using both longitudinal epidemiological methods and the genetically-sensitive twin design. Specifically, I sought to i) compare weight gain trajectories in Gemini across one of the most detailed measures of IFMs to date, ii) explore the potential mechanisms that places formula fed infants at greater risk of RIWG, and iii) remove residual confounding from shared environmental exposures in the relationship between IFMs and infant weight gain trajectories in the first year of life.

Study 2; Parent-Child Reciprocity in Infant Feeding Modalities and Infant Weight Development Across the First Year of Life sought to test the presence of parent-child reciprocity in infant feeding within the Gemini sample. Leveraging both bi-directional epidemiological analysis and the twin design, the specific aims were to i) measure whether the introduction of formula milk is a response to slower weight gain or concern for slower weight gain during the early weeks of infancy, ii) explore whether the introduction of formula milk influences weight gain trajectories after its introduction, and iii) explore whether early weight gain or concern for weight gain leads to twin-pair discordance in infant feeding.

In the final study of part of the thesis; Study 3; Gene-Environment Interplay in Infant Feeding Modalities and Infant Weight Development, I again use the twin design to explore both GE correlation and GE interaction between IFMs and infant weight gain. Specifically, I explored i) whether caregivers introduce formula milk in *response* to their infant's genetic liability towards rapid weight gain in the first few weeks of life (GE Correlation) and ii) whether parental provision of formula milk provides greater propensity for expression of genetic liability towards RIWG in the first year of life (GE interaction)

Study 4; A Digital Intervention to Promote Responsive Formula-Feeding and Healthy Growth in Infancy; a Protocol for the BRIGHT Intervention presents the development of a digital prototype for an intervention seeking to support health and overall well-being in families that use formula milk in the UK (0-1 y) – the Baby Responsive Intervention for Growth and Health Tracking (BRIGHT). The BRIGHT resources will sit within the established and widely used Baby Buddy app. Given my thesis' focus on milk-feeding within infancy, this chapter will present the BRIGHT resources related to milk feeding – including four 'modules' on formula feeding, growth monitoring, sleep, and crying – which I led. These were developed using the Person Based Approach (PBA) for Intervention Development and insights from Chapters 3, 4 and 5 were applied to help shape BRIGHTs focus on responsive parenting.

Chapter Two. Methods for Part One of Thesis

2.1 Description of the Gemini Twin Cohort for Studies 1 to 3

The Gemini twin study was established in 2007 and set out to investigate genetic and environmental influences on weight development in early childhood.[121] Gemini is richly phenotyped for infant growth, infant & child appetite, parental feeding methods and practices, as well as measures of the home family environment. Crucial to this thesis, Gemini contains detailed information regarding infant feeding methods and behaviour in the exclusive milk feeding period, making it an ideal source to explore both environmental and genetic influences of infant weight patterning. The baseline sample includes 2402 families with twins (n=4,804 singletons) born in England or Wales in 2007 who consented to take part. Participants were recruited by letter from the Office for National Statistics inviting eligible families from birth registration data to participate.

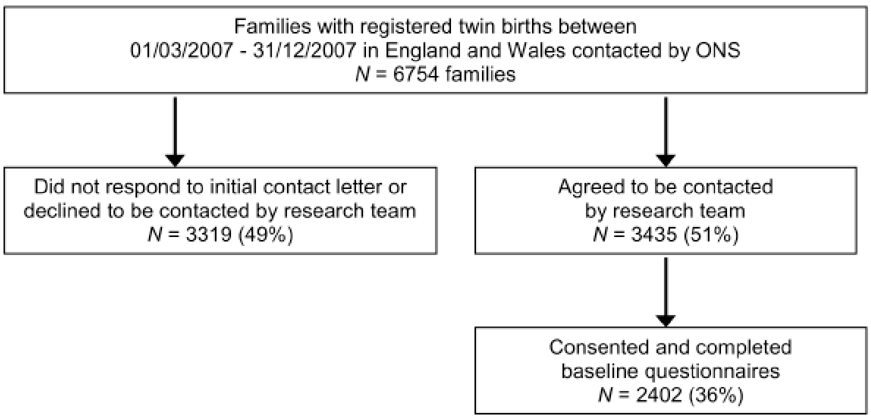


Figure 2.1. Flow diagram of recruitment of Gemini Families obtained from van Jaarsveld et al[121]

The geographical distribution of Gemini families mirrors that of the UK population.[121] Moreover, the sample is representative of UK twins on both sex and zygosity distribution, gestational age at birth, and birthweight.[121] At baseline, the sample consisted of 749 monozygotic (MZ) twin pairs and 1,616 dizygotic (DZ) twin pairs, with 37 pairs of unknown zygosity.

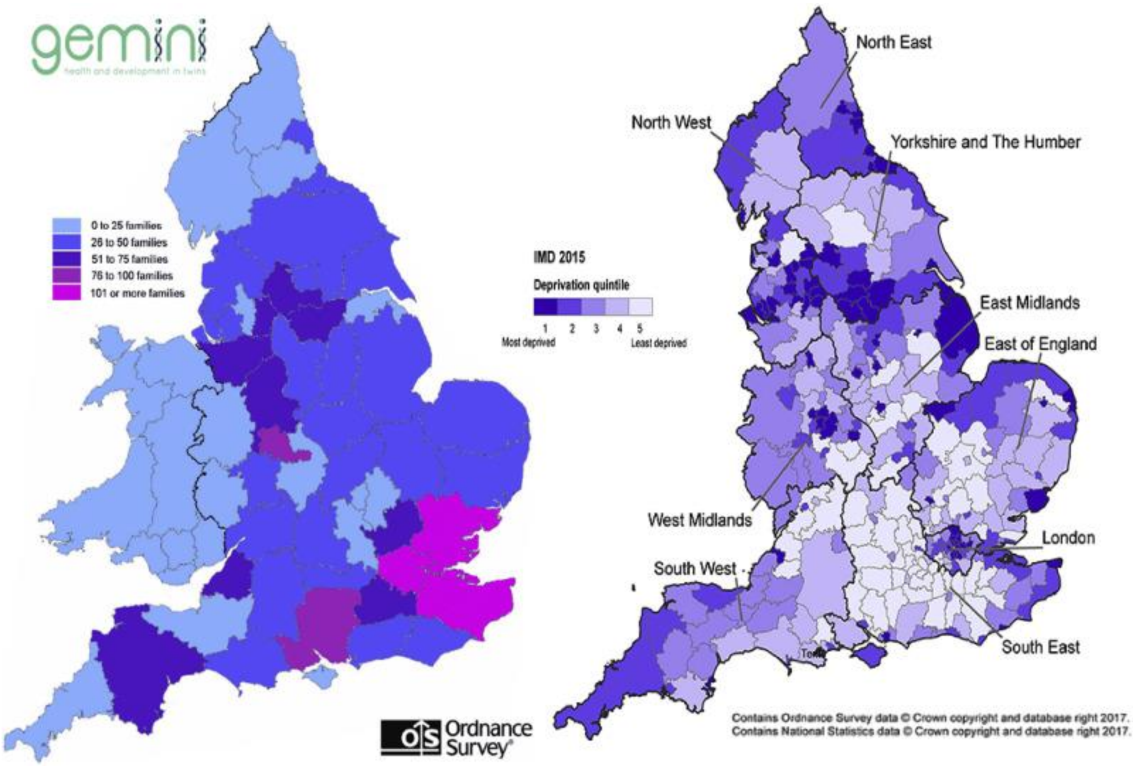


Figure 2.2**.** Distribution of Gemini Twins across the United Kingdom shown in the map on the left (adapted from van Jaarsveld et al, 2010), while the map on the right shows the level of deprivation within the United Kingdom based on the index of Multiple Deprivation (Reproduced from the Department for Communities and Local Government, 2015) – obtained from Kininmonth et al[122]

Key Measures from Gemini used in this Thesis

Various measures from the; Baseline (at ~8-months of age) questionnaire and 16-month questionnaire, were utilised for the current thesis. Each of these measures is outlined in **Figure 2.3**. Baseline measures were taken from mother-reported questionnaires completed at baseline when the twins were approximately 8 months of age, and which included many aspects of infant feeding from birth, that were reported retrospectively. Consenting families were then followed up at regular intervals across infancy, toddlerhood and childhood (e.g. 8, 16, 21 months). Each of these measures is described in detail throughout this chapter. Together, the Gemini Twin Study provides an ideal cohort to explore bio-psycho-social interactions in infant feeding and weight development, as it contains the most comprehensive infant feeding and growth data of any UK-based twin cohort to date.

T0 - 8 months old		T1 – 16 months old	
Latent Variable	**Measure**	**Latent Variable**	**Measure**
Infant Feeding Modality and Milk Feeding Practices	Infant Feeding Questionnaire and New Items Developed for Gemini	Weight Gain Trajectories	Birthweight, and all weight measurements collected after birth up to 15-months of age
Maternal Concern for Low Weight Gain	Adapted from the Child Feeding Questionnaire	Rapid Infant Weight Gain	Birthweight, and all weight measurements collected after birth up to 15-months of age
Infant Feeding Practices	Adapted Infant Feeding and Child Feeding Questionnaire and New Items Developed for Gemini		
Weight Gain Trajectories	Birthweight, and all weight measurements collected after birth up to 8-months of age		
Rapid Infant Weight Gain	Birthweight, and all weight measurements collected after birth up to 8-months of age		
Sociodemographic Variables	Various Items in Baseline Questionnaire		

Figure 2.3. Measures utilised across Studies 1-3 from Gemini Twin Study[121]

2.2. Descriptive Epidemiology of Infant Feeding Modalities in Gemini

Information regarding infant feeding practices, representing both the type of milk (i.e. breastmilk vs. formula milk) and modalities (i.e. bottle-feeding expressed breast milk vs. breastfeeding from the breast) was collected in the Baseline Questionnaire when infants were ~8-months of age (SD = 2.2 months, range 4.0–20.3 months). For each of these items, mothers were asked to think back to their twins' first three months of life, to report on milk-feeding behaviours before the introduction of solid foods. Infant feeding modality was assessed using two separate items: 'Which feeding methods did you use in the first three months?'. Response options were: 'entirely breastfeeding'; 'mostly breastfeeding with some bottle-feeding'; 'equally breastfeeding and bottle-feeding'; 'mostly bottle-feeding and some breastfeeding'; 'almost entirely bottle-feeding (only tried breastfeeding a few times)'; 'entirely bottle-feeding (never tried breastfeeding)'; and 'other, please describe'. Mothers provided a response separately for each twin. It was explained that 'breast-feeding' referred to feeding an infant with breast milk, either directly from the breast or expressed milk from a bottle, while bottle-feeding referred to formula milk given from a bottle. Hence, 'expressed feeding' was reported as breastfeeding, as opposed to bottle-feeding. A second item was therefore used to clarify the specific *modality* of breastfeeding used, if mothers reported having breastfed their infant. Hence, apart from mothers who responded with 'entirely bottle-feeding', mothers were asked 'What was your main method of breastfeeding?', with response items including: 'Mostly fed directly from the breast'; 'Equally fed from the breast and given expressed breast milk'; and 'Mostly given expressed breast milk'. 3,382 (70.39%) of mothers from the baseline sample provided responses to the first general question, and 2,455 (51.10%) provided responses to the more detailed second question on breastfeeding. Responses from these two items were combined to form the broad 3-item infant feeding method measure, and more detailed 7-item infant feeding modality measure. Whilst the priority of the study was to develop a more detailed measure of infant feeding than has been used in past research, the 3-group measure was also used to allow comparisons between the current study with previous research.

The distribution of responses from mothers to these two items are displayed in **Table 2.1.**

Table 2.1. Distribution of Feeding Methods Across Feeding Modalities in Gemini Twin Baseline Sample (n=4,804)

	Infant Feeding Method[a]							
Method Of Breastfeeding[b]	Entirely breastfeeding	Mostly breast some bottle	Equally breast and bottle	Mostly bottle some breast	Almost entirely bottle	Entirely Bottle	Other[c]	Total
Directly from Breast	543 (80.33%)	642 (71.73%)	231 (51.79%)	328 (41.89%)	270 (39.36%)	-	69 (35.20%)	2083 (43.65%)
Equally from Breast and Expressed	45 (6.66%)	106 (11.84%)	101 (22.65%)	158 (20.18%)	76 (11.08%)	-	17 (8.67%)	503 (10.54%)
Expressed	66 (9.76%)	141 (15.75%)	111 (20.18%)	282 (36.02%)	198 (28.86%)	-	74 (8.67%)	872 (18.27%)
True Missing	22 (3.25%)	6 (0.67%)	3 (0.67%)	15 (1.92%)	142 (20.70%)	1090 (100%)	36 (18.37%)	1,314 (27.54%)
Total	**676 (100%)**	**895 (100%)**	**446 (100%)**	**783 (100%)**	**686 (100%)**	**1090 (100%)**	**196 (100%)**	**4,804 (100%)**

[a]Mothers asked at 8-months; Which feeding methods did you use in the first three months?

[b]Mothers asked at 8-months; what was your main method of breastfeeding in the first 3 months? (if not entirely bottle-feeding)

[c]Other: Represents a response option ‘other’ where mothers were mothers were asked to ‘describe their feeding method’ where this option was selected. These responses represent responses which could not be attributed to the appropriate Infant Feeding Method and Method of Breastfeeding based on mothers open text-box responses.

-; Missing as mothers did not respond to Method of breastfeeding if Infant was Entirely Bottle-fed

Removal of Pre-Term Infants

Infants born prior to 36 weeks (gestational age in weeks <36) were excluded from the main analyses in Studies 1 and 2 to reduce potential confounding and bias introduced by infants born 'pre-term'. This cut off was chosen in keeping with the timing of sucking reflex development, at 34-35 weeks of age, which is likely to influence infant feeding exposures and was in keeping with previous analyses of the Gemini Study.[91,121] Pre-term infants are more likely to receive early specialist care in hospital, experience feeding complications regarding breastfeeding initiation, and be born at low birthweight[123]. Both of these exposures have potential to influence both the feeding modality through which an infant is fed, as well as their weight gain trajectories, thereby confounding the relations of interest between infant feeding modalities and growth outcomes such as RIWG. Moreover, given the use of a twin sample, birthweights and gestational age are lower than singleton births. For instance, 29.89% of the Gemini Twins were born <36 weeks, whereas figures the UK Office For National Statistics estimates the prevalence of pre-term births to be 7.8% across the population.[25] Hence, the exclusion of pre-term infants – ranging from extremely preterm (<28 weeks) to moderately pre term (≤35 weeks) helps to increase the generalisability of the sample to the wider population of infants in the UK. The final sample eligible for analysis contained 3402 individual twins from 1701 families - 70.75% of consenting families who completed the baseline questionnaire.

2.2.1 Infant Feeding Method: Broad 3-Group Measure

First, I sought to stratify term Gemini infants into a broader infant feeding method, similar to measures used in past literature. This measure consisted of three groups; exclusively breastfed infants, mixed-fed infants, and predominantly formula fed infants. Infants fed primarily with breastmilk either directly from the breast or from expression were placed together into the 'breastfed' group. The distribution of infants across these groups are presented below in **Table 2.2.** 3158 of 3402 (92.83%) term Gemini infants had sufficient data to be included in this measure; 473 (13.90%) were categorized as 'breastfed'; 1495 (43.94%) were categorised as 'mixed fed'; 1190 (34.98%) were categorized as 'formula fed'; and 244 (7.17%) were missing data on infant feeding methods.

2.2.2 Infant Feeding Modality: 7-Group Measure

A more detailed infant feeding modality measure was generated using the same two items from the baseline questionnaire. This measure sought to stratify term Gemini infants by both infant feeding methods (breastfeeding vs. formula feeding) and infant feeding modality (e.g. breastmilk from the breast vs. breastmilk from a bottle) To categorise infants, the *primary* method of feeding was used, as opposed to the *exclusive* method of feeding. In other words, infants were placed in their infant feeding modality group based on the main source of infant feeding. A more stringent approach favouring exclusivity would have resulted in a larger number of small groups (i.e., exclusive expressed feeders as opposed to predominantly expressed feeders; n=14, 0.005% of eligible sample as opposed to n=58, 1.70%), which would not have carried sufficient analytical power to test associations particularly after restraining the sample to those with sufficient weight data. This approach was adopted for all groups apart from exclusively breastfed infants fed directly from the breast, to provide an exclusively breastfed reference group for the analyses. Overall, this variable included seven infant feeding modalities: exclusively breastfed; breastfed and expressed fed; expressed fed; breastfed and formula fed; breastfed, formula fed and expressed fed; formula fed and expressed fed; and predominantly formula fed infants. As demonstrated in **Table 2.2**, the exclusively breastfed group included infants whose mothers responded that they were both ‘entirely breastfeeding’ and ‘Mostly fed directly from the breast’ at 3 months of age (n=448, 13.17%). Breastfed and expressed fed infants were either ‘entirely breastfeeding’ or ‘mostly breastfeeding, some formula feeding’ with equal amounts from breast and expressed milk (n=77, 2.26%). Expressed fed infants were either ‘entirely breastfeeding’ or ‘mostly breastfeeding, some formula feeding’ and mostly given expressed breast milk (n=58, 1.70%). It was decided that this ‘expressed’ group would represent both exclusively and predominantly expressed fed infants, as compared to only exclusively expressed fed infants. Given the low number of exclusively expressed fed infants born ≥36 weeks (n= 14), this group would be too small to power associational analyses in infants with RIWG data at 3-months (n=14) and 12-months (n=8) of age. The breastfed and formula fed infants were fed either ‘mostly breast some formula’, ‘equally breast and formula’, ‘mostly formula, some breast’ with breastmilk coming directly from the breast (n=1058, 31.10%). The breastfed, expressed fed, and formula fed infants were either fed ‘equally breast and formula’ or ‘mostly formula, some breast’ but with breastmilk coming equally from breast and expression (n=165, 4.85%), thereby representing predominantly mixed feeders who were fed with a range of feeding modalities. The expressed fed and formula fed infants were fed either ‘equally breast and formula’ or ‘mostly formula and some breast’ whether breastmilk was provided through expression (n=162, 4.76%). Finally, as within the infant feeding method variable described above, predominantly formula fed infants were fed either ‘almost entirely formula’ or ‘entirely formula’ during the first 3-months of life (n=1190, 34.98%). Upon inspection of the timing of formula milk introduction, reported through the item “How soon after birth did you start bottle-feeding your twins?” the vast majority of formula fed infants were given formula at less than or one day after birth n=867 (78.75%); with the remaining 198 (17.98%) being introduced to formula between 1 day and 1 week after birth. Therefore, it was deemed appropriate these infants were representative of having been predominantly formula fed across the first 3-months of life, as most received breastmilk for less than a day. Overall, 244 (7.17%) infants were missing information on infant feeding modality.

Table 2.2. Description of Derived Infant Feeding Modality Measures Across Infant Feeding Methods and Method of Breastfeeding in Gemini Infants Born ≥36 weeks (n=3158)

	3-Group Measure of Infant Feeding Modalities (n=3158)			**7-Group Measure of Infant Feeding Modalities (n=3158)**		
	Method of Breastfeeding (B8)[b]			**Method of Breastfeeding (B8)[b]**		
Infant Feeding Method (B5)[a]	Directly from Breast	Equally from Breast and Expressed	Expressed	Directly from Breast	Equally from Breast and Expressed	Expressed
Entirely breastfeeding (n=646)	Group 1: Breastfed (n=448)	Group 1: Breastfed (n=11)	Group 1: Breastfed (n=14)	Group 1: Exclusively Breastfed (n=448)	Group 2: Breastfed and Expressed Fed (n=11)	Group 3: Expressed Fed (n=14)
Mostly breast some formula (n=889)	Group 2: Mixed Fed (n=557)	Group 2: Mixed Fed (n=66)	Group 2: Mixed Fed (n=44)	Group 4: Breastfed and Formula Fed (n=557)	Group 2: Breastfed and Expressed (n=66)	Group 3: Expressed Fed (n=44)
Equally breast and formula (n=443)	Group 2: Mixed Fed (n=200)	Group 2: Mixed Fed (n=70)	Group 2: Mixed Fed (n=47)	Group 4: Breastfed and Formula Fed (n=200)	Group 5: Breastfed, Expressed Fed and Formula Fed (n=70)	Group 6: Expressed Fed and Formula Fed (n=47)
Mostly formula some breast (n=768)	Group 2: Mixed Fed (n=301)	Group 2: Mixed Fed (n=95)	Group 2: Mixed Fed (n=115)	Group 4: Breastfed and Formula Fed (n=301)	Group 5: Breastfed, Expressed Fed and Formula Fed (n=95)	Group 6: Expressed Fed and Formula Fed (n=115)
Almost entirely formula (n=544)	Group 3: Formula Fed (n=247)	Group 3: Formula Fed (n=63)	Group 3: Formula Fed (n=98)	Group 7: Formula Fed (n=247)	Group 7: Formula Fed (n=63)	Group 7: Formula Fed (n=98)
Entirely formula (n=1090)	Group 3: Formula Fed (n = -)	Group 3: Formula Fed (n = -)	Group 3: Formula Fed (n = -)	Group 7: Formula Fed (n=-)	Group 7: Formula Fed (n=-)	Group 7: Formula Fed (n=-)

[a]Mothers asked at 8-months; ‘Which feeding methods did you use in the first three months?’ for both their 1st and 2nd born twin. Breast feeding was described as any method of breastfeeding, including expressed milk from the bottle, where Bottle-Feeding was described as using formula milk through a bottle

[b]Mothers asked at 8-months; what was your main method of breastfeeding in the first 3 months? If they were not entirely bottle-feeding their infants

- Indicates that mothers did not respond to breastfeeding method if they had previously indicated they were feeding using ‘entirely bottle-feeding’

2.2.3 Infant Feeding Modality Discordance for Chapter's Three and Chapter Four

2.2.3.1 Infant Feeding Modality Discordance

For Studies 1 and 2 I derived a measure of IFM discordance to capture discordant infant feeding across the Gemini sample. The 7-group infant feeding modality measure, detailed under the previous section 2.2.2 was used to define the discordance samples for the DT analyses. Where co-twins differed on this 7-group measure, in any combination of varied feeding methods, they were labelled as discordant. This resulted in a sample of n=139 term (≥36 weeks gestation) twin pairs discordant for feeding modalities, to any extent across any of the seven possible categories (8.86% of Gemini term twin pairs). Within this sample, I sought to identify the co-twin fed with a *higher risk* feeding modality. This consisted of infants being fed from either more expressed breastmilk from a bottle or more formula feeding than their co-twin, in line with previous literature highlighting bottle-feeding as a potential mechanism for RIWG.[50] To do this, I allocated a score for each of the 7 IFM groups, with a lower score representing more breastfeeding from the breast and a higher score indicating more bottle-feeding – either from expressed breastmilk or formula feeding. Specifically, Exclusively Breastfed Infants = 1, Breastfed and Expressed Fed = 2, Expressed Fed = 3, Breastfed and Formula Fed = 4, Breastfed, Formula Fed and Expressed Fed = 5, Formula Fed and Expressed Fed = 6, and Primarily Formula Fed = 7. Hence, each co-twin was given a score based on the IFM category they fell under, and the twin with the higher 'score' was labelled as fed with 'higher risk feeding modality'. In scenarios where co-twins were fed with equal levels of bottle feeding but from different types of milk, such as 'Breastfed and Expressed Fed' vs. 'Breastfed and Formula Fed" the infant fed with more formula milk was labelled as higher risk. Each of these decisions is outlined below Table 2.3. Overall, this method allowed us to identify twin pairs where one twin was fed through a more 'high risk' feeding modality than their co-twin. This broad risk-based approach allowed us to retain as much twin-pair discordance on IFMs as possible, given the relatively small number of twins discordant for IFMs in Gemini. This was therefore deemed the most appropriate and feasible approach to explore whether weight gain trajectories vary across infant feeding modalities under Study 1.

2.2.3.2 Formula Feeding Discordance for Chapter Four

In chapter four, we undertook a DT analysis examining discordance in terms of formula feeding specifically in line with the studies aims. Hence, where co-twins differed on the IFM variable and one twin was fed specifically with more formula feeding than their co-twin (i.e. exclusively breastfed vs. breastfed and formula fed), the twin pair was labelled discordant. Twin pairs which varied in IFM but not such that one twin was fed with more formula milk (i.e. breastfed vs. breastfed and expressed fed) were labelled as concordant. This resulted in a small sample of n=62 discordant twin pairs (4% of eligible term sample). In a similar process to that described above, the twin who was fed with more formula feeding than their co-twin was labelled as 'fed with more formula-feeding'. This discordance categorisation is described in **Table 2.3**.

Table 2.3. Definition of Twin Discordance Across Term Gemini Twin Pairs (≥36 weeks)

Twin Pair Discordance in Infant Feeding Modality (7-group measure)		Definition of Discordance		Definition of Higher Risk Twin		N of discordant twin pairs (% of all discordant pairs)
Twin 1	Twin 2	Discordant on Infant Feeding Modality (Chapter Three)	Discordant on Formula Feeding (Chapter Four)	Twin Fed with Higher Risk Feeding Modality (Chapter Three)	Twin Fed with More Formula Feeding (Chapter Four)	
Exclusively Breastfed	Breast and Expressed Fed	Y	N	Twin 2	-	8 (5.76%)
Exclusively Breastfed	Expressed Fed	Y	N	Twin 2	-	7 (5.04%)
Expressed Fed	Breast and Expressed Fed	Y	N	Twin 1	-	2 (1.44%)
Breast and Formula Fed	Breast, Formula, and Expressed Fed	Y	N	Twin 2	-	19 (13.67%)
Breast and Formula Fed	Formula and Expressed Fed	Y	N	Twin 2	-	28 (20.14%)
Formula and Expressed Fed	Breast, Formula, and Expressed Fed	Y	N	Twin 1	-	13 (9.35%)
Breast and Expressed Fed	Breast and Formula Fed	Y	Y	Twin 2	Twin 2	6 (4.32%)

Breast and Expressed Fed	Breast, Formula, and Expressed Fed	Y	Y	Twin 2	Twin 2	2 (1.44%)
Breast and Expressed Fed	Formula Fed	Y	Y	Twin 2	Twin 2	1 (0.72%)
Breast and Formula Fed	Breast and Expressed Fed	Y	Y	Twin 1	Twin 1	8 (5.76%)
Breast and Formula Fed	Exclusively Breastfed	Y	Y	Twin 1	Twin 1	17 (12.23%)
Formula Fed Only	Breast and Formula Fed	Y	Y	Twin 1	Twin 1	13 (9.35%)
Breast and Formula Fed	Expressed Fed	Y	Y	Twin 1	Twin 1	5 (3.6%)
Exclusively Breastfed	Formula and Expressed Fed	Y	Y	Twin 2	Twin 2	4 (2.88%)
Formula and Expressed Fed	Formula Fed	Y	Y	Twin 2	Twin 2	3 (2.16%)
Formula Fed	Breast, Formula, and Expressed Fed	Y	Y	Twin 1	Twin 1	1 (0.72%)
Formula Fed	Exclusively Breastfed	Y	Y	Twin 1	Twin 1	2 (1.44%)
Total						139

Infant Feeding Modalities; Exclusively Breastfed: Fed exclusively breast milk from the breast; Breastfed and Expressed Fed: Fed with a combination of breast milk from the breast and expressed milk in a bottle; Expressed Fed: Fed with expressed milk in a bottle; Breastfed and Formula fed; Fed with a combination of breast milk from the breast and formula milk; Breast, Formula and Expressed: Fed with a combination of breast milk from the breast, expressed milk in a bottle, and formula milk; Formula and Expressed Fed: Fed with a combination of expressed milk from a bottle and formula milk; Formula Fed: Fed with formula milk

2.3 Descriptive Epidemiology of Infant Weight Development in the Gemini Twin Study

2.3.1 Description of Infant Anthropometric Data

Across the Gemini Cohort, mothers were asked to report weight measurements extracted from infant health records where possible, a process that has shown adequate accuracy when compared to clinical measurements.[124] Majority of these weights were made by health professionals, however parents were also asked to send in additional weight measurements every three months, taken by weighing scales provided when the children were about 2 years old.[121] 96.4% of weight measurements at 3 months in Gemini had been taken by a healthcare professional as compared to parent-reported measurements of weight. Weight measures from both the baseline questionnaire at ~8-months and the T1 at ~15 months were utilised to derive measures of infant weight development. Across 4804 infants; parents reported a mean of 6 weight measurements by 6-months, and 11.5 (Inter Quartile Range 8-15) weight measurements by 16-months of age[52]. This raw weight data was cleaned in 2010, such that impossible valuables were removed following the individual examination of individual growth trajectories for all 2402 Gemini twin pairs. Any outliers were assessed against the original questionnaires (if available) for data entry errors and corrected where possible. Measurements deemed to be a clear error in measurement or deemed impossible were recoded to missing.

2.3.2 Approaches to Measuring Rapid Infant Weight Gain

Rapid Infant Weight Gain (RIWG) is defined as an infant experiencing an increase of >0.67 in their weight-for-age z-score (weight z-score) between two timepoints during infancy, equivalent to an upward crossing over a single centile band on the UK-WHO Growth Charts.[12] Weight z-scores are used to assess RIWG instead of raw weight (in kg) as they account for the infant's age and sex, which are crucial.[22] Therefore, in the present thesis RIWG is represented in two ways; i) trajectories of rapid weight gain (using repeated measures of weight for age z-score) and ii) a continuous measure of change in weight-for-age z-score from birth to 6- or 12-months of life where trajectories could not be modelled. For Studies 1 and 2 I will focus largely on the former longitudinal measures of rapid growth, represented by trajectories of weight for age z-scores across the first year of life. Hence, the Gemini infants' weight measurements, alongside the infants' gestational age (in weeks) and sex, were used to calculate a weight-for-age z-score at each possible point in infancy using the STATA zanthro package in line with the UK-WHO Growth References.[125] Whilst the UK-WHO Growth References do not consider gestational age in deriving z-scores for babies born to term (≥37 weeks), for babies born pre-term (<37 weeks) z-scores are derived using gestationally corrected age (age of infant minus number of weeks born before term) in line with the UK-WHO Growth standards. Hence, when deriving the UK-WHO z-scores using the zanthro package, two separate commands were used to derive z-scores for term infants (≥37 weeks gestation), UKWHOterm, and pre-term infants (<37 weeks), UKWHOpreterm. Using these repeated measures of weight for age z-scores, trajectories of weight for age z-scores, in line with the UK-WHO Growth References, will be modelled using linear mixed effects models (LMMs). These methods were chosen as the primary outcome variable, in favour of single crude measures of RIWG – such as presence of RIWG (yes/no), as such measures may be subject to error given the highly variable nature of infant growth across infancy. This also allowed for the repeated weight data in Gemini to offer the analyses greater power as well as offer a more nuanced measures of rate of infant weight gain. However, where trajectory analyses were not possible under the research questions outlined – such as under the use of the Logistic Regression Models to measure the likelihood of formula milk introduction (Study 2) and Univariate Twin Models (Study 3) – I chose to use the continuous measure of RIWG (change in weight-for-age z-score from birth to 12-month) over the categorical measure of RIWG (experiencing RIWG vs. not). Once again, this continuous measure – whilst only containing two weight measurements - allows for a more nuanced understanding of weight gain than a categorical measure of RIWG. For example, where two infants may both experience RIWG at 12-months (increase of >0.67 in their weight-for-age z-score from birth to 12-monnths), they may in fact show quite distinctive patterns of weight gain. One infant may show a change z-score of 1.33 (a two-centile upward crossing) from birth to 12-months, whilst the other shows a change z-score of 0.68 (a single upward centile crossing). Similarly, across two infants who did 'not' experience RIWG, one may have shown weight faltering with a decrease in change z-score of >-0.67, whilst another remained close their centile line with an increase of 0.11 change z-score. In this way, a continuous measure of RIWG was favoured where modelling trajectories of growth was not possible.

2.3.2.1 Change in Weight-for-Age Z-Scores

The rich set of repeated weight measurements available, as described above, were used to generate change in weight for age z-score from birth to 12-months of age Once again, the UK-WHO Growth Standards were used, adjusting for age of weight measurement, sex, and gestational age of the infant (for pre-term infants), using the zAnthro growth macro package in Stata [125] Change in weight for age z-scores (change z-scores) were specifically calculated by subtracting an infant's birth z-score from their weight for age z-score at the outcome timepoint of interest – such as at 12-months.

2.3.2.2 Trajectories of Weight-for-Age Z-Scores

The rich source of longitudinal weight data in Gemini was further utilised to model trajectories of weight-for-age z-scores where possible. Hence, the calculated weight-for-age z-scores at each possible point in infancy were utilised to model trajectories of z-scores. This allows for examination of how infants 'track' in their weight gain as compared to the reference population, accounting for their gestational age and sex. The calculation of these Linear Mixed Effects Models (LMMs) is presented below in section 2.4.1.

2.4 Between Families Observational Analyses and Methods in The Gemini Twin Study

Between family models were utilised to explore relationships between IFMs and infant weight gain in studies 1 and 2. These models utilise data from both twins as independent data points, however clustering within families is adjusted for. In Study 1, LMMs were undertaken. Whilst in Study 2, both Splined LMMs and Logistic Regression Models were undertaken.

2.4.1 Linear Mixed-Effects Models (LMMs)

For Study One (Aim 3.1), LMMs with maximum likelihood estimation were used to estimate the association between a i) 3-group infant feeding method variable and ii) 7-group infant feeding modality variable and weight-for-age z-score trajectories to 12-months of age. Maximum likelihood estimation was employed such that individual twins who had a sufficient number of z-scores – five z-score measurements from baseline (~3 weeks) to 52 weeks of age (~12 months) – were included. This aligns with similar procedures for previous studies examining infant growth trajectories.[50,70] The LMMs modelled z-score trajectories from the second occasion of a weight measurement (mean = 3.18 weeks of age) as opposed to birth to enhance the model fit. As weight change in the first two to three weeks of life is highly variable, including weights within this period led to higher residuals and poorer fit in prediction of weight gain trajectories to 12-months of life. Therefore, it was deemed appropriate for these models which were concerned with longitudinal weight gain trajectories to 12-months to exclude the first weight measurement. Nonetheless, weight-for-age z-score at birth was included in the final model as a covariate to adjust for the influence of birthweight over infant weight gain trajectories. The respective models included: baseline z-score to represent difference in weight z-scores between exposure groups at baseline (~3-weeks), as well as the interaction between the exposure and a linear time term (i.e. IFMxAge) and quadratic time term (i.e. IFMxAge^2) to represent differences in *rate of weight gain* across IFM groups to 12-months of age. Time was represented using both a linear (age) and quadratic (age squared) term to allow for a non-linear trend in growth and improving the fit of the growth trajectories. The Akaike information criterion (AIC) and Bayesian information criterion (BIC) were used to determine model fit. Finally, the LMMs were adjusted for the set of prespecified covariates as described in section 2.44 as well as clustering of twins within families to enable both twins in a pair to be included in analyses. Analyses were conducted in Stata version 16[126]

2.4.2 Splined Linear Mixed-Effects Models (Splined LMMs)

Under Study Two (Aim 4.2), Splined LMMs with MLE were used to predict weight z-score trajectories prior to and following the introduction of formula milk between two IFM groups; i) Infants EBF to ~3-months of age ii) infants EBF to 2 weeks of age and then introduced to formula milk (either in combination with breastmilk or in isolation). The 'spline' was centred at the point of formula introduction (0), such that estimates were generated for the period 'before' (-6 to 0 weeks) and 'after' the introduction of formula (0 weeks to ~52 weeks). This 'spline' was centred at 6 weeks for EBF, infants not introduced to formula milk, to offer a comparison to EBF infants. 6-weeks was chosen as the most common week of formula introduction for those infants introduced to formula. To generate z-score trajectories plots, the association between the periods 'before' and 'after' formula introduction on z-score trajectories was modelled separately for both infants EBF and infants fed with formula milk. First, to explore differences in weight for age z-score trajectories between IFM groups *before* the introduction of formula milk, an interaction term between the 'week prior the introduction of formula milk' and 'IFM group' was added to a LMMs model. Then, to explore differences weight for age z-score trajectories between IFM groups *after* the introduction of formula milk, an interaction term between the 'week following the introduction of formula milk' and 'IFM group' was added to a separate LMMs model. β values (with 95% CI's) were used to estimate the mean difference in z-score slope between EBF and formula fed infants in these two periods. Statistical significance was set at .05 and we report results in full to focus on effect sizes rather than p-values. Akaike information criterion (AIC) and Bayesian information criterion (BIC) were used to determine model fit. A pre-specified set of covariates were adjusted as described in section 2.4.4, as well as clustering within families. Analyses were conducted in Stata version 16[126]

2.4.3 Logistic Regression Models

Logistic regression models were utilised under Study 2 (Aim 4.1) to test the hypothesis that i) lower change z-score (continuous) from birth to the week in which formula milk was introduced (or 6-weeks for EBF infants) or ii) maternal concern for low weight gain between 0-3 months (binary; yes, no) would predict a higher odds of being introduced to formula milk (binary; yes, no). Models were fitted using maximum likelihood estimation (MLE) and model fit was evaluated using Akaike information criterion (AIC) and Bayesian Information Criterion (BIC). Models were adjusted for the covariates specified and clustering within families. Statistical significance was set at $p<0.05$ and placed emphasis on effect sizes rather than p-values. Analyses were conducted in Stata version 16[126]

2.4.4 Approach to The Selection of Covariates for Observational Models

The selection of covariates is crucial to between family models – as important variations between families (e.g. income or social deprivation) may confound observational associations of interest. The inclusion of covariates in the models was based on theoretical evidence for relevance to either infant feeding practices or infant growth outcomes. Theoretical relevance was prioritised, as opposed to demonstrated associations within the present sample, such that if a certain covariate was theoretically related to the variables of interest but showed no association in the current sample using the current measures, it was still included in the final adjusted models. This approach was chosen as variables may not be statistically significant in the current sample if regression coefficients are under or overestimated.[127,128] In other words, merely selecting covariates with small p-values, in contrast to a more theoretical approach, carries the risk of including only overestimated coefficients. This would run the risk of not adjusting for important exposures, which might explain observed associations between infant feeding practices and RIWG. Moreover, it allows for easier comparisons with previous studies that have controlled for theoretically important variables, potentially undetected in the current sample. However, to consider over-adjustment and the inclusion of potential collider variables, unadjusted models are presented in supplementary files for relevant analyses throughout the thesis and potential colliders are named and removed from final models where relevant. Finally, it is of note that given the simultaneous measurement of the IFMs, infant growth, and covariates of interest at ~8-months (baseline in Gemini Sample) the loss of sample to non-reporting of covariate data was minimal across the current observational models.

A number of covariates were considered for inclusion in the between family models, including both perinatal and postnatal infant characteristics and family demographics. Covariates were identified based on previous literature demonstrating evidence of associations with either infant feeding method (e.g. breastfeeding), or infant growth outcomes. Infant-related covariates included: birthweight z-score[129]; zygosity (dizygotic or monozygotic); twin sex; gestational age (in weeks) self-reported by mothers at the baseline questionnaire; days spent in specialist care (0 days; 1-4 days; 5-9 days; 10 or more days), self-reported by mothers for each twin in the baseline questionnaire. The timing of introduction to solid foods was measured by the question: ‘ How old were the twins the very first time solid foods of any kind were eaten (i.e. anything other than milk)?‘ provided in either weeks or months (<4 months; 4 to <5 months; 5 to <6 months; $\geq$6months). Maternal and family-level covariates included: age at delivery (in years), self-reported by mothers at the baseline questionnaire; mode of delivery (caesarean or vaginal) self-reported by mothers at baseline; maternal BMI (kg/m2), calculated using maternal self-reported weight and height in the baseline questionnaire; maternal ethnicity (white or non-white collapsed due to small numbers; but originally 16 groups); maternal marital status at baseline (married; divorced or separated; single); maternal smoking during pregnancy (no; yes, indicative of *any* smoking during the pregnancy period); parity (none; one; more than one) reported at baseline; the presence of gestational diabetes self-reported at baseline (no; yes). A composite latent measure of socioeconomic status (SES) was also included in the final models. This latent measure represents an aggregate of several individual, family and community level indicators of SES. More specifically, this measure aggregated: Maternal Educational Qualification; Household National Statistics Socio-economic Classification (NS-SEC) indicating the household reference persons socio-economic position based on their occupation and other job characteristics base; Home Ownership Status; Gross Annual Household Income; Index of Multiple Deprivation; Number of bedrooms; and Number of Cars. Each of these measures was collected at the baseline round of data collection when twins were ~8 months of age. For further information detailing the construction of the aggregate SES variable see Kininmonth et al[122]

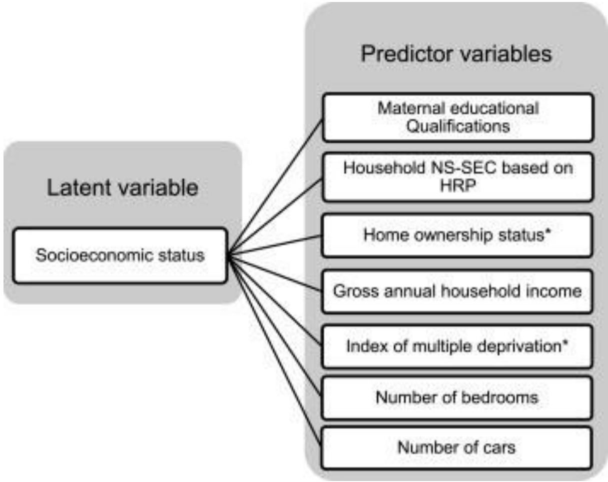


Figure 2.4. The indicators of socioeconomic status that were included within the composite measure of SES in Gemini obtained from Kininmonth et al[122]

2.4.5 Statistical Significance

For observational analyses described above and the twin models described below, an alpha $p < .05$ was considered statistically significant. We did not adjustment for multiple testing in line with the Gemini study policy.[121] Rather we report results in full (estimates with 95% CIs), with the focus on effect sizes rather than p-values.

2.5 The Twin Design and Methodology for Chapter Four

2.5.1 The Twin Design

Twin pairs have long been leveraged as a natural experiment to explore the relative contribution of genetic and environmental influences over a trait or phenotype of interest. The classical twin design is a valuable tool for teasing apart the contribution of genetic and environmental factors to variation in a measured phenotype. The basis of the method is to compare similarity between Monozygotic twin pairs (who share nearly 100% of genetic material) to that between DZ twin pairs (who share ~50% of their segregating genetic material).[35] As both types of twin pairs primarily share their environments, insofar as they are born to the same household and the same socioeconomic positioning, a greater similarity in a trait between MZ than DZ pairs suggests a genetic contribution to variation in a measured phenotype (e.g. RIWG). The bigger the difference in similarity, the greater the genetic contribution. It is estimated using the statistic 'heritability'.[84] On the other hand, if both types of twins are highly similar, this indicates strong influence from aspects of the environment that twin pairs share entirely. The extent to which MZ pairs are not correlated indicates the influence of environmental factors unique to each individual twin (not shared with their co-twin), because these are the only factors that can explain differences between identical twin pairs.

For decades, researchers have leveraged the twin design to estimate the relative importance of genetic and environmental factors to the development of important diseases, behaviours, and health outcomes in both adulthood and childhood.[84,130] It is worth mentioning here that heritability estimates do not model the strength of genetic influence on a trait itself (e.g. BMI), but rather the variation in that trait in the population (e.g. variation in BMI). This means that heritability can naturally vary across populations if the variation of that trait varies, and this needs be considered when interpreting heritability estimates. Nonetheless, the twin design can leverage co-variance between twins in numerous ways to explore genetic and environmental influences over a trait, garner more causal inference in relationships between traits of interest, as well as explore complex interactions between genes and the environment – or GE interplay. Each of these applications will be described with respect to the thesis below.

2.5.2 Zygosity

To establish zygosity in the Gemini baseline questionnaire, opposite-sex twins were classified as DZ. Then, mothers of same-sex twins were asked to complete a validated 18-item zygosity questionnaire for young twins to establish whether the pair was DZ or MZ. This questionnaire has been validated using DNA markers, showing agreement for 95% of cases, and has shown substantial reliability over time.[131] Nonetheless, a subset of mothers in Gemini repeated this same questionnaire when the twins were 29 months of age (n=934, 58.9% of baseline sample).[132] Both these reports were validated with a DNA test with a random sample of 81 Gemini pairs, where the DNA results exactly matched the results of the questionnaire in all cases.[132] DNA was additionally used to classify pairs could not be classified using the zygosity questionnaire.

2.5.3 Univariate Twin Models

Using twin comparisons as described above, a univariate twin model can explore genetic and environmental contributions to variation in a single trait of interest (e.g. RIWG). The first step of univariate twin models explores twin pair similarity between MZ and DZ pairs using intraclass correlations. These patterns of MZ and DZ similarity provide an indication of the relative contribution of three different sources of variance in the measured phenotype: additive genetic influences or 'heritability' (A); shared environmental influences (C); and unique or non-shared environmental influences, which also includes random measurement error (E). Falconer's formula can be used to estimate the magnitude of A, C and E: A is estimated by doubling the difference between the MZ and DZ correlation (A = (rMZ – rDZ) * 2); C is estimated by subtracting A from the MZ correlation (C = rMZ – A); E is the remaining variance not explained by A or C and is estimated by 1.0 minus the MZ correlation (because the rMZ = (1.0 * A) + (1.0 * C).[133]

Maximum likelihood structural equation modelling (MLSEM) is then used to provide more reliable estimates with 95% confidence intervals as well as goodness-of-fit statistics for different types of models. MLSEM uses path analysis to model A, C and E, using the same principles as Falconer's equations, which are based on the extent to which MZ and DZ pairs share genetic and shared environmental variation.[134] Genetic correlations between twin pairs are fixed at 1.0 for MZs and 0.50 for DZs. Shared environmental correlations between twin pairs are the same for MZs and DZs, because they are exposed to the same household and wider social environment, so this is fixed at 1.0 for both. First, a saturated model is used to fit the data with no parameter constraints, such that only means, covariances, and variances for MZ and DZ twins are estimated separately. Then goodness-of fit statistics are used to examine whether the full ACE model fits the data well compared to this saturated model. Thereafter, nested submodules with fewer parameter estimates can be tested to see if a more parsimonious and simpler model can be fit to the data. For example, a nested CE model drops A, a nested AE model drops C, and a nested E model drops A and C. These nested submodules are tested against the full ACE model using goodness of fit statistics, such as the Akaike's Information Criterion (AIC = chi-square - 2 df) with lower estimates indicating a better fit. Similarly, the Likelihood Ratio Test (LRT) describes the change in the chi-squared test P value between models – with a non-significant P value indicating no significant improvement in model fit.

2.5.4 Bivariate Twin Model Fitting

The univariate model can be extended to a bivariate or multivariate model, to estimate both the univariate A, C and E parameters underlying each phenotype, as well as the extent to which multiple phenotypes share their A, C and E influences.[134] Bivariate twin models were intended to be used within Study Three to explore whether IFMs and RIWG to 3-months of age share common A, C and E influences. To estimate the extent to which the same A, C and E influences underlie two traits, *cross-twin cross-trait correlation*s (CT-CT) across the two phenotypes of interest (e.g. IFM and RIWG) are compared for MZ and DZ pairs – e.g. Twin 1's IFM is correlated with Twin 2's RIWG, and Twin 1's RIWG is correlated with Twin 2's IFM. As with the univariate models, the pattern of CT-CT correlations provides an indication of the extent to which common A, C and E influences explain the phenotypic correlation between them. For example, a greater CT-CT correlation between MZs than DZs indicates that common genetic influences underlie the phenotypic correlation between them; on the other hand, a high CT-CT correlations for both MZ and DZ pairs indicates that the same shared environmental influences an important in explaining the observed phenotypic correlation between them.

Similar to the univariate model, MLSEM BT models can then decompose variation in IFMs and RIWG into the previous A, C and E estimates, as well as other statistics that indicate the extent of common genetic and environmental influence - **etiological correlations** and **bivariate estimates** of A, C, and E.[135,136] **Etiological correlations** represent the extent to which the genetic or environmental influences underlying IFMs and RIWG are the same or different, and include: the additive genetic correlation [rA], shared environmental correlation [rC], and non-shared environmental correlation [rE]. They range from –1.0 to 1.0 and can be interpreted like a Pearson's correlation – e.g. a higher positive genetic correlation indicates that a large proportion of the genetic factors underlying one trait also underlie the other, while a correlation close to 0 indicates very few common genetic factors. **Bivariate estimates** of A, C and E quantify the proportion of the phenotypic association between two phenotypes that can be explained by genetic (bivariate A), shared environmental (bivariate C), or non-shared environmental (bivariate E) influences. These models produce goodness-of-fit statistics such as the AIC and Likelihood Ratio Test (LRT) to indicate the most parsimonious final model., testing whether bivariate configuration fit the data well as compared to the fully saturated model All models will be undertaken usen OpenMX version 2.2.6 in R.[136]

2.5.5 Heterogeneity Univariate Twin Models (GE Interaction Models)

The univariate ACE Model can also be extended to generate a heterogeneity univariate ACE model – often called a GE interaction model, to test for differences in the magnitude of A, C and E estimates for variation a trait of interest, between subgroups. This model was used to test for differences in A, C and E estimates for RIWG from 0-12 months, between IFM groups (breastfed; mixed fed; and formula fed) in Study Three.

Once again, two methods are used to estimate variation in A, C and E influences across the groups of interest: i) twin correlations and ii) MLSEM. First, twin pair correlations were calculated for MZs and DZs, stratified by each IFM group (breastfed, mixed-fed, formula fed). Then, a heterogeneity (interaction) twin model (called a 'common effects model') was used to test for differences in parameter estimates (A, C, and E) across the three IFM groups. 95% CIs for the parameter estimates and goodness-of-fit statistics were also generated. The heterogeneity model tests 3 models for goodness-of-fit. First a full common effects model is run which allows parameter estimates for A, C and E to differ across the 3 IFM groups, as well as the variances for infant weight gain. Then, a more constrained scalar model is run and compared to the common effects model, which allows for variance differences in infant weight gain between the IFMs groups, but constrains the parameter estimates for A, C and E to be the same across groups. Lastly, a fully constrained model (null model) is run and compared to the scalar model, which neither allows A, C or E parameter estimates or variances to differ across the IFM groups (and equates to a standard univariate model). If, using the AIC and Likelihood Ratio Test (LRT), the scalar model shows no significant reduction in fit compared to the common effects model (GE model), this would indicate that there are no differences in variance differences in change z-score across the three IFMs groups. If the null model shows no reduction in fit compared to the common effects model this will indicate that there is no difference in the parameter estimates for A, C or E across the IFMs groups.

2.5.6 The Discordant Twin (DT) Design

The DT design takes advantage of MZ and DZ twin pairs who show differences – or who are *discordant* – on an exposure or trait of interest.[35,83,137] If twin pairs are discordant on a trait of interest (e.g. IFM), one can explore whether this difference may lead the twins to differ on an outcome of interest (e.g. RIWG), whilst removing confounding from all unmeasured shared environmental influences, because twin pairs are matched entirely for many environmental factors). The DT design offers a more powerful approach than unrelated individuals, for studying the association between an exposure of interest and an outcome, in cases where both are strongly socially patterned (e.g. breastfeeding and rate of infant weight gain). In unrelated samples, differences in the shared environment and varying genetic liability between individuals can confound an association of interest. For example, breast-fed infants may show more favourable weight gain due to their caregiver's higher social positioning and the wide-reaching benefits that affords them (such as a higher quality diet during complementary feeding or early childhood). DT comparisons offer a valuable counterfactual design in this scenario, to remove the influence of social positioning. For example, if one DZ co-twin had been exclusively breastfed while their co-twin was breastfed and fed with formula milk – any differences in growth could not attributed to environmental factors that were shared by twin pairs – such as social positioning. Therefore, if an association between breastfeeding and infant weight gain is observed in unrelated individuals but not replicated among DZ twin pairs, this would imply that the association is the sample of unrelated individuals is contributed to by confounding by factors shared by twin pairs. Given the social patterning of infant feeding behaviours[42,43], where higher sociodemographic families are more likely to breastfeed their infants, removing these confounding influences is important, yet relatively rare in this field of research.

Discordant Twin comparisons can be conducted in multiple ways. Firstly, one can use inferential statistics – such as LMMs – to explore whether growth trajectories vary between discordant co-twins. These methods were employed in Study One. Moreover, one can also explore whether continuous or categorical variables of interest – such as anthropometric variables like change z-scores – vary across discordant twins using regression models (for continuous measures) or logistic regressions (for categorical measures) such as in Study 2. Both approaches can adjust for non-shared covariates which might vary across co-twins and perhaps be associated with discordance and/or the outcome of interest – such as specialist care in early infancy or differences in birthweight.

However, it is important to consider the limitations and considerations when making DT comparisons. Firstly, whilst DT models remove influences from shared environmental exposures, non-shared environmental exposures and differences between twins can still bias DT comparisons. Hence, it is imperative to reflect on *why* twin pairs may be discordant on a trait of interest, as there may be important reasons for discordance that need be considered. This is particularly important in infancy, where discordance on any measured variable – such as feeding or weight development – is likely to be highly meaningful. For example, one twin may experience an illness that leads to discordance in feeding or weight gain – this experience may therefore confound associations of interest between feeding and weight gain. Hence, it is imperative to consider how differences across the twin pairs may influence relationships of interest – just as much as wider social influences might. Whilst this can be a limit to DT comparisons, this consideration can also be leveraged to identify non-shared environmental characteristics which might lead to co-twin discordance. For example, differences in a non-shared characteristics (e.g. differences in anthropometrics) between co-twins discordant for feeding can be explored to pinpoint potential reasons *why* twins are fed differently in infancy. In this way – the DT design can be particularly useful tool to research parent-child reciprocity in early feeding experiences if applied mindfully.

Secondly, statistical power is lesser within fixed-effects analyses, as compared to mixed-effects - through which DT comparisons are made.[138] Therefore, caution needs to be applied when interpreting DT results, with consideration of the more imprecise estimates that these methods offer. Nonetheless, the discordant twin design is a powerful and promising approach that is able to: i) cast a light on associations after removing confounding from social and economic factors and garner more causal inference and ii) highlight non-shared environmental or infant characteristics that might lead to discordance in an outcome. These applications are particularly useful for exploring infant feeding interplay, as gold-standard evidence from randomized control trials is difficult to develop in this research field, yet confounding from social patterning presents a major challenge to epidemiological studies of infant feeding and infant weight gain.

Chapter Three. Study 1: Infant Feeding Modalities and Infant Weight Gain Trajectories Across the First Year of Life

3.1 Introduction

Accumulating evidence suggests that experiencing RIWG during the first year of life is consistently associated with overweight and obesity in later childhood and adulthood.[18,19] Given these findings, there has been increasing attention on infancy as a key period to establish healthy weight development.[9] Within this period, milk feeding is presumed to be the critical, if not causal, influence over infant weight development.[139] In the UK, both formula feeding (feeding exclusively with formula milk) and mixed feeding (feeding with a combination of breastmilk from the breast, breastmilk expressed into a bottle, and formula milk) are common. Whilst 55% of mothers in the UK Infant Feeding Survey report mixed-feeding at 6-weeks of life[32], few observational studies have sought to disentangle the prevalence of RIWG across diverse combinations of mixed-feeding. Therefore, the first aim of this chapter is to investigate infant weight gain trajectories across a detailed 7-groups measure of IFMs. Moreover, I also compare weight gain trajectories between a broader 3-group infant feeding method variable – breastfed infants, mixed-fed infants, and formula fed infants – to facilitate comparison between Gemini data and findings from other cohorts.[53,54]

Investigating infant growth outcomes between the detailed IFMs measure also provides an opportunity to investigate potential mechanisms which may place formula fed infants at greater risk of RIWG.[52,53,140] Whilst few studies have examined mechanisms that may lead to rapid growth in formula fed infants, most propose that rapid growth is attributable to the varying nutritional makeup of formula versus breastmilk.[109] However, it remains possible that behavioural differences in feeding that occur when feeding through a bottle and not the breast might also explain the increased risk of RIWG for formula fed infants. To help disentangle these two potential mechanisms, the current study will compare weight gain trajectories between i) expressed fed infants (fed with predominantly expressed breast milk from a bottle) to ii) infants exclusively breastfed from the breast within the first ~3 months of life. If expressed fed infants show more rapid growth than infants fed breastmilk from the breast, then bottle feeding may contribute to rapid growth. To date, few studies have contained sufficiently detailed data on infant feeding modalities to tease apart these potential mechanisms – the bottle or the milk – which places formula fed infants at greater of RIWG.

Another important limitation of research within this field is the use of cross-sectional measures of RIWG, such as weight change from birth to either 6-or 12-months of age.[12] Hence, evaluating IFMs in relation to weight gain *trajectories*, using repeated measures of weight across the first year of life, would provide important new insights. Crucially, cross-sectional measures of RIWG may mask important but temporary variations in weight gain patterns.[41] Given the rich set of anthropometric data captured by the Gemini study, longitudinal weight trajectories were modelled to explore trajectories of rapid weight gain across one the most detailed measure of IFMs to date.

Finally, given the ethical implications of 'randomising' infants to receive either breast or formula milk, literature on infant feeding modalities and weight development has relied largely on observational studies. As such, associations between infant feeding and growth outcomes may be confounded by a range of social, behavioural, and genetic factors. As detailed in the literature review – the pervasive social patterning of breastfeeding behaviour[42,43] is particularly critical to consider as a potential confounder in the relationship between formula feeding and rapid growth. In other words, the benefits of breastfeeding on infant growth may be a proxy for the benefits afforded by socio-economic advantage – such as higher quality diet during complementary feeding or more social support. Therefore, the following chapter will utilise the twin design of Gemini, using a Discordant Twin (DT) analysis, to remove residual confounding from the social patterning of breastfeeding. In short, comparing weight gain trajectories among twin pairs who fed discordantly, such that one twin received a higher risk feeding modality (either more expressed breastfeeding or formula feeding) can help to better remove confounding from environmental factors shared by twins – such as their socioeconomic positioning.

3.2 Aims

The overall objective of chapter three was to explore whether a range of infant feeding modalities are associated with weight gain trajectories across the first year of life in the Gemini Study. To achieve this objective, I triangulate between-subjects methods with the DT design to i) compare weight gain trajectories across one of the most detailed measures of IFMs to date ii) explore the potential mechanisms that places formula fed infants at greater risk of RIWG and iii) remove residual confounding from shared environmental exposures in the relationship between IFMs and infant weight gain trajectories. The specific aims and research questions are outlined below.

Aim 3.1: Explore variations in infant weight gain trajectories across the first year of life between a broad (3-group) and detailed measure of Infant Feeding Modalities (7-group)

RQ 1: Do weight gain trajectories to 12-months of age vary across a broad 3-group measure of Infant Feeding Modalities; breastfed, mixed-fed and formula fed infants?

- ➔ *Hypothesis: Weight gain trajectories to 12-months of age will be steeper for mixed fed and formula fed infants, as compared to exclusively breastfed infants*

RQ 2: Do weight gain trajectories to 12-months of age vary across the full range of feeding modalities: exclusively breastfed (from the breast); breast and expressed fed; exclusively expressed fed; breast and formula fed; breast, formula and expressed fed; formula and expressed fed; and formula fed infants?

- ➔ *Hypothesis: Weight gain trajectories to 12-months of age will be steeper for infants fed with either a mixture of expressed feeding and formula-feeding or breastfeeding and formula feeding, as compared to exclusively breastfed infants*

Aim 3.2: Explore mechanisms that may promote rapid weight gain in formula fed infants; the bottle versus formula milk

RQ 3.: Do weight gain trajectories to 12-months of age vary between i) exclusively breastfed infants (infants who are exclusively breastfed from the breast)? ii) expressed fed infants (infants who are breastfed from a bottle) and iii) formula fed infants?

- ➔ *Hypothesis: Weight gain trajectories to 12-months of age will be steeper for both expressed fed infants and formula fed infants, as compared to exclusively breastfed infants (fed from the breast)*

Aim 3.3: Apply the Discordant Twin (DT) design to remove social confounding from associations between IFMs and infant weight gain trajectories across the first year of life

RQ 4: Do twin pairs discordant for IFMs show varying weight gain trajectories to 12-months of age?

- ➔ *Hypothesis: Twins discordant for IFMs will not show significantly different weight gain trajectories to 12-months of age*

3.3 Methods

3.3.1 Measures

3.3.1.1 Infant Feeding Modalities

Information on infant feeding methods was collected when infants were ~8-months (M=8.2 SD=±2.2 months). For the following analyses, the infant feeding exposure variables (IVs) were used i) a broad 3-group Infant Feeding Modality Variable and ii) a more specific 7-group Infant Feeding Modality variable (IFMs) described **Figure 3.1.** The methods used to derive these variables is described in detail in Chapter Two, Section 2.2.

IFM Exposure Variable	Definition or Categories
Broad Infant Feeding Modality (3-groups)	1. Exclusively Breastfed (ref) 2. Mixed Fed 3. Formula Fed
Detailed Infant Feeding Modalities (7-groups)	1. Exclusively Breastfed (ref) 2. Breastfed and Expressed Fed 3. Expressed Fed 4. Breastfed and Formula Fed 5. Breastfed, Formula Fed, and Expressed Fed 6. Formula Fed and Expressed Fed 7. Formula Fed

Figure 3.1. Infant Feeding Modality (IFM) Variables in the Gemini Twin Study

3.3.1.2 Infant Weight Gain Trajectories

In the baseline questionnaire (completed when twins were approximately 8-months of age) and the 16-months questionnaire, parents were asked to copy all weight measurements taken from birth, and the date of each measurement, from each of their twin's personal child health record. This resulted in a rich source of longitudinal weight data, with parents reporting a median of 6 weight measurements by 6-months, and 11.5 (Inter Quartile Range 8-15) weight measurements by 16-months of age[52] inclusive of their birthweight. Weight-for-age z-scores (weight z-score) were then calculated for each weight measurement provided, using the UK-WHO Growth Standards, adjusting for the age at each weight measurement, sex, and gestational age of the infant, in line with the WHO recommendations. The zAnthro growth macro package in Stata was used for these calculations[125] Mean weight z-scores (at 0, 3, 6 and 12 months) across IFMs will first be presented descriptively. Them, these weight z-scores will be used to derive weight gain trajectories using linear mixed-effects models (LMMs) with maximum likelihood estimation. For further details of the methods employed to derive these weight gain trajectories see Section 2.4.1 of Chapter Two.

3.3.1.3 Infant Feeding Modality Discordance

The 7-group infant feeding modality measure, detailed under Section 2.2.2 of Chapter Two was used to identify twin pair discordance in infant feeding modality. The methods through which twin pairs discordant for IFM are also outlines in Section 2.2.3 of Chapter Two. In summary, the co-twin who was fed through a *higher risk* IFM – was identified as the 'high risk' twin, alongside their lower risk co-twin. In this context, the higher risk twin was defined as having been given more expressed breastmilk from a bottle or formula milk. This allowed us to compare weight gain trajectories between discordant twin pairs in a meaningful manner

3.3.1.4 Covariates

When exploring weight gain trajectories between IFMs, both relevant perinatal and postnatal infant characteristics related to either IFMs or weight development, were included in the adjusted models. This selection procedure and the details of measurement are as described in section 2.4.4. Overall, these Covariates included; birthweight z-score, zygosity, twin sex; gestational age, days spent in specialist care, timing of introduction to solid foods, maternal age at delivery, mode of delivery, maternal BMI, maternal ethnicity, maternal marital status, maternal smoking during pregnancy, the presence of gestational diabetes and a composite measure of socioeconomic status. Moreover, the models adjusted for clustering within families.

When exploring weight gain trajectories between twins discordant for IFMs, non-shared environmental factors and individual level factors that have the potential to differ between twin pairs, were adjusted for in the DT models. These included twin pair *differences* in the variables reported in the baseline questionnaire including; sex; birthweight; days spent in specialist care; and age at introduction to solid foods. The continuous difference between co-twins (e.g., birthweight z-score: 0.32) or binary difference (e.g. sex difference: 0=same-sex; 1=different sex) were then added to the adjusted DT models.

3.3.2 Samples

The current chapter utilised data from the Gemini Twin Study, as described in Section 2.1. Infants born <36 weeks gestational age were excluded from the current analytic sample (n= 1382 of 4804 infants, 28.76% of baseline sample). These infants were removed to reduce potential confounding and bias introduced by infants born before pre-term as described in section 2.1.

The final sample for the adjusted weight z-score trajectories between both the 3-group and 7-group IFM variable, included 1998 individual twins. These included individual Gemini Twins with reported measures of Infant Feeding Modality (3-group or 7-group measure), sufficient weight data to model weight z-score trajectories (five z-scores measurements from baseline (~3 weeks) to 52 weeks of age (12 months) and respective covariates. For the third aim, weight z-score trajectories were then compared across term twin pairs with reported discordance on IFMs reported at ~3-months of age, as described above. This resulted in a sample of n=106 twin pairs (8.86% of the consenting families who completed the baseline questionnaire) discordant for IFMs and with sufficient weight data and covariates for the DT analyses.

3.3.3 Statistical Analysis

3.3.3.1 Aim 3.1: Explore variations in infant weight gain trajectories across the first year of life between a broad (3-group) and detailed measure of Infant Feeding Modalities (7-groups)

Linear mixed-effects models (LMMs) with maximum likelihood estimation were used to estimate the association between the i) 3-group IFM and ii) 7-group IFM variable (categorical IVs) on weight-for-age z-score trajectories from ~3 weeks to 12-months of age, adjusting for covariates listed above and clustering within families. Models are described in Chapter Two Section 2.4.1. The respective models included: baseline weight-for-age z-score (at ~3.18 weeks) to represent differences between IFM groups at baseline; and the interaction between infant feeding modality groups and a linear time term (IFMxAge) and a quadratic time term (IFMxAge^2) on weight-for-age z-score, to represent differences in trajectories between IFM groups from baseline to 12-months of age. Akaike information criterion (AIC) and Bayesian information criterion (BIC) were used to determine model fit. Analyses were conducted in Stata version 16[126]and a $P < .05$ was considered significant. β values (with 95% CIs) estimate the mean difference in weight-for-age z-score (per week) between each of the IFM groups as compared to exclusively breastfed infants as the reference group, at both baseline and across the 12-month period using the linear (IFMxAge) and quadratic interaction term (IFMxAge^2).

3.3.3.2 Aim 3.2: Explore mechanisms that may promote rapid weight gain in formula fed infants; the bottle versus the formula milk

LMMs with maximum likelihood estimation were used to compare weight gain trajectories, derived under aim one, between i) EBF (infants who are exclusively breastfed from the breast) ii) expressed fed infants (infants who are primarily breastfed from a bottle) and ii) formula fed infants. The methods for deriving these weight gain trajectories are described above and in Chapter Two, Section 2.4.1.

3.3.3.3 Aim 3.3: Apply the Discordant Twin (DT) design to account for social confounding from associations between IFMs and infant weight gain trajectories across the first year of life

For the DT analysis, LMMs with maximum likelihood estimation were used to model continuous differences in weight gain trajectories between twin pairs (MZ and DZ pairs) who were discordant for IFMs, had sufficient weight data (≥5 measurements), and were born ≥ 36 weeks gestation. The LMM model predicted the z-score trajectory from ~3 weeks to 52 weeks of age for the twin who was fed with a higher risk feeding modality as compared to their co-twin fed with a lower risk feeding modality, adjusting for the covariates described above. Time was represented using both a linear (age) and quadratic (age squared) term to allow for a non-linear trend in growth and improving the fit of the growth trajectories. Model fit was determined by the AIC and BIC.

3.4 Results

3.4.1 Descriptives of Anthropometrics Across Feeding Modalities in Gemini

Prior to the application of LMMs to predict inferential weight gain trajectories between IFMs, we present and plot the mean weight-for-age z-scores at 0, 3, 6, 9 and 12 months between the broad 3-group more specific 7-group IFMs variable for Gemini infants born ≥36 weeks of age where weight data was available. These plots are presented as a description of anthropometrics in the Gemini sample.

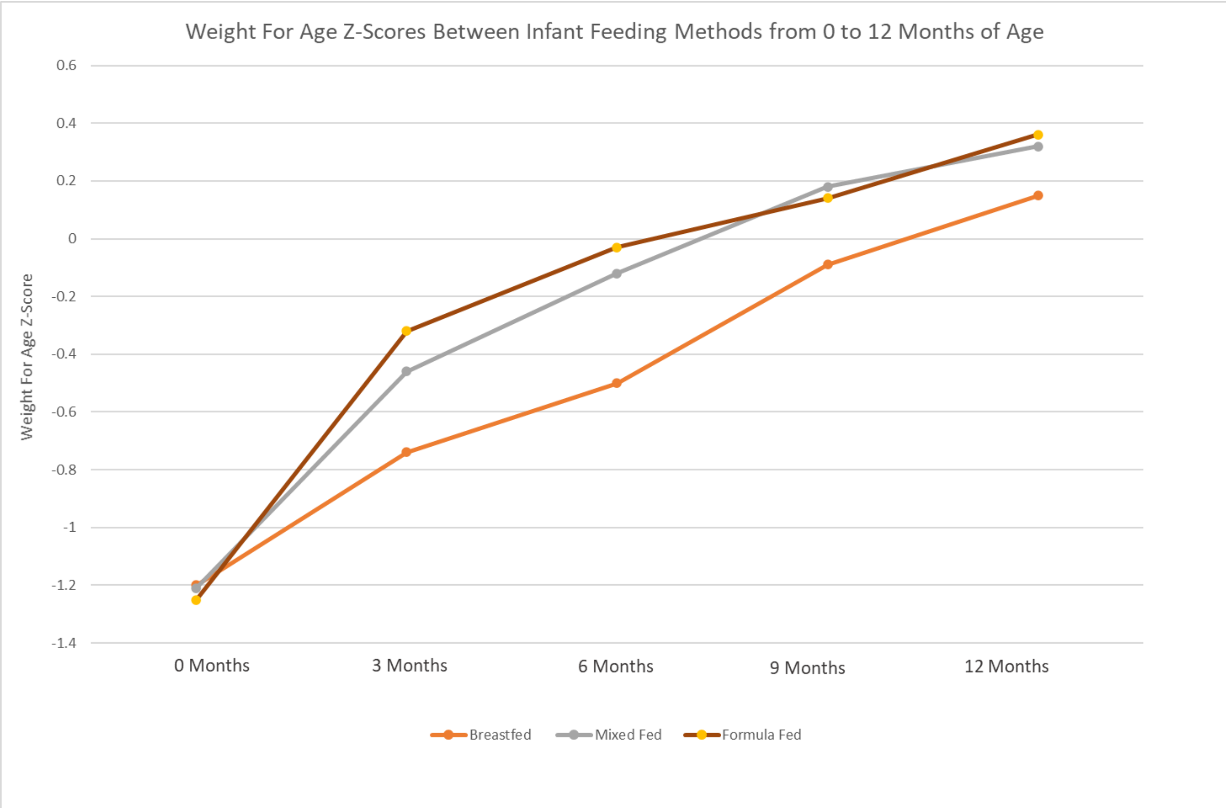
On average, the Gemini twins were born at relatively low birthweight relative to the UK-WHO reference population, irrespective of infant feeding method. For instance, EBF (n=447) were born at a mean z-score of -1.19, equating to the 11th centile on the UK WHO growth charts.[141] Moreover, the twins did not show relatively large z-scores across the first 12-months, with the IFM group with the highest average z-scores at 12-months; breast, formula and expressed fed infants falling on the 68th centile (n=161, mean z=score=0.48). **Figures 3.2 and 3.3** descriptively presents the mean z-score between infants across the broad 3-level and detailed 7-level IFMs variables.

Table 3.1. Mean Weight for Age Z-scores Across Infant Feeding Modalities in Term Gemini Infants (≥36 weeks)

	Weight for Age Z-Score									
	0 Months		3 Months		6 Months		9 Months		12 Months	
	N	*M (± SD)*	*N*	*M (± SD)*	*N*	*M (± SD)*	*N*	*M (± SD)*	*N*	*M (± SD)*
Infant Feeding Modality[a] (3-groups)										
Exclusively Breastfed	472	-1.20 (0.86)	443	-0.74 (1.04)	401	-0.50 (0.99)	315	-0.09 (0.96)	274	0.15 (1.03)
Mixed Fed	1,454	-1.21 (0.86)	1,387	-0.46 (1.13)	1,205	-0.12 (1.03)	953	0.18 (0.98)	789	0.32 (0.98)
Formula Fed	1,167	-1.25 (0.97)	1,062	-0.32 (1.04)	910	-0.03 (1.08)	665	0.14 (1.00)	500	0.36 (1.06)
Infant Feeding Modality[b] (7-groups)										
Exclusively Breastfed	447	-1.19 (0.85)	419	-0.73 (1.03)	377	-0.52 (0.99)	299	-0.11 (0.96)	262	0.13 (1.02)
Breastfed and Expressed Fed	77	-1.39 (0.91)	73	-0.76 (1.05)	66	-0.39 (0.99)	60	-0.03 (1.04)	40	0.11 (1.13)
Expressed Fed	58	-1.19 (1.03)	56	-.54 (1.35)	53	-.16 (1.05)	36	0.08 (1.03)	38	0.17 (1.05)
Breastfed and Formula Fed	1,025	-1.18 (0.82)	975	-.45 (1.13)	852	-.08 (1.03)	673	0.21 (0.98)	546	0.36 (0.97)
Breast, Formula Fed and Expressed Fed	161	-1.12 (0.99)	154	-.25 (1.09)	127	-.06 (1.09)	92	0.31 (0.98)	88	0.48 (0.96)
Formula and Expressed Fed	158	-1.41 (0.92)	153	-0.60 (1.07)	131	-0.30 (0.97)	108	0.03 (0.93)	89	0.07 (0.98)
Formula Fed	1,167	-1.25 (0.97)	1,062	-0.32 (1.04)	910	-0.03 (1.08)	665	0.14 (1.00)	500	0.36 (1.06)
Total	3093		2892		2516		1933		1563	

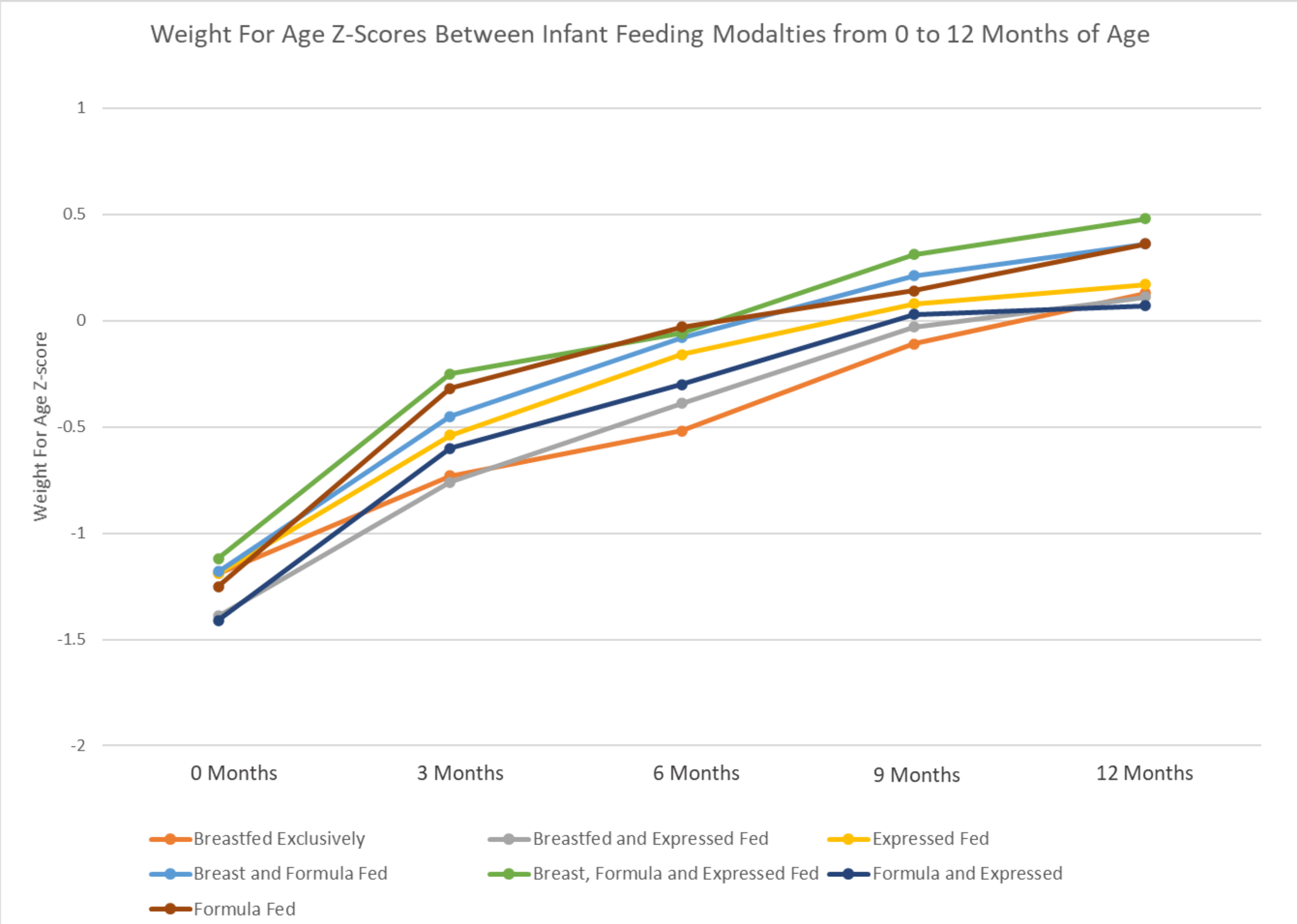
Weight for Age Z-Scores: Calculated using UK-WHO growth refence data adjusting for age, sex, and gestational age.
[a]Exclusively Breastfed: Fed breast milk from the breast or from expressed milk; Expressed Milk Fed; Fed with primarily expressed milk from a bottle or equal amount of breastmilk from the breast and expressed milk from a bottle; Mixed-Fed: Fed with a combination of breast milk from the breast, expressed milk in a bottle, and formula milk; Formula Fed: Fed with formula milk
[b]Exclusively Breastfed: Fed exclusively breast milk from the breast; Breastfed and Expressed Fed: Fed with a combination of breast milk from the breast and expressed milk in a bottle; Expressed Fed: Fed with expressed milk in a bottle; Breastfed and Formula fed; Fed with a combination of breast milk from the breast and formula milk; Breast, Formula and Expressed: Fed with a combination of breast milk from the breast, expressed milk in a bottle, and formula milk; Formula and Expressed Fed: Fed with a combination of expressed milk from a bottle and formula milk; Formula Fed: Fed with formula milk

Figure 3.2. Mean Weight for Age Z-score Trajectories from 0 to 12-Months of Age Across IFMs (3-groups)



Weight for Age Z-Scores: Calculated using UK-WHO growth refence data adjusting for age, sex, and gestational age.
Exclusively Breastfed (n=472): Fed breast milk from the breast or from expressed milk; Mixed-Fed (n=1454): Fed with a combination of breast milk from the breast, expressed milk in a bottle, and formula milk; Formula Fed (1167): Fed with predominantly formula milk

Figure 3.3. Mean Weight for Age Z-score Trajectories from Birth to 12-Months of Age Across IFMs (7-groups)



Weight for Age Z-Scores: Calculated using UK-WHO growth refence data adjusting for age, sex, and gestational age.
[b]Exclusively Breastfed (n=487): Fed exclusively breast milk from the breast; Breastfed and Expressed Fed (n=77): Fed with a combination of breast milk from the breast and expressed milk in a bottle; Expressed Fed (n=58): Fed with expressed milk in a bottle (n=1025); Breastfed and Formula fed (n=161); Fed with a combination of breast milk from the breast and formula milk; Breast, Formula and Expressed (n=158): Fed with a combination of breast milk from the breast, expressed milk in a bottle, and formula milk; Formula and Expressed Fed (n=162): Fed with a combination of expressed milk from a bottle and formula milk; Formula Fed (n=1167): Fed with predominantly formula milk

3.4.2 Aim 3.1: Explore variations in infant weight gain trajectories across the first year of life between a broad (3-group) and detailed measure of Infant Feeding Modalities (7-groups)

3.4.2.1 Sample Characteristics

Table 3.2 presents the descriptive characteristics of the Gemini baseline sample, alongside the analytic subsamples of infants included in the LMMs (n=1998, 41.59% of eligible infants). In regard to the broad 3-group IFM, the distribution of EBF (baseline; 14.90%, analytic; 15.87%), mixed feeding (baseline; 47.86%, analytic; 48.40%) and formula feeding (baseline; 37.24%, analytic; 15.87%) was similar across the analytic and baseline sample. Similarly, the distribution of infants across the more detailed 7-group IFM measure was similar, with the largest proportion of infants being formula fed (baseline; 37.24%, analytic; 35.74%), followed by breastfed and formula fed (baseline; 27.37%, analytic; 33.73%), and exclusively breastfed (total; 12.37%, analytic; 14.86%) to 3-months of age. The prevalence of RIWG was similar across samples, with approximately half of infants (baseline; 44.85%, analytic; 55.14%) experiencing RIWG (change in weight for age z-score > 0.67) from 0-3-months. Prevalence of RIWG from 0-12 months was high, with most infants experiencing RIWG (baseline; 66.41%, analytic; 79.03%).

Across both samples, most twin pairs were dizygotic (baseline: 66.33%; analytic: 70.77%), spent 0 days in specialist care (baseline: 76.32%; analytic; 80.63%) and were born at about 37.5 weeks gestation (baseline: 36.20 weeks; analytic: 37.43 weeks). Mothers had a similar average BMI (baseline: 25.10; analytic: 24.94), were largely of white ethnicity (baseline: 92.96%; analytic: 94.59%), and around half were first time mothers (baseline: 51.42%; analytic: 52.15%), with about ~10% of mothers smoking during pregnancy (baseline: 11.17%; analytic: 9.61%). Across both samples, the majority of families were of a high socioeconomic position, indexed using NS-SEC (baseline: 64.84%; analytic: 68.07%). Similarly, a small proportion of families had a household income in the lowest band of <22.5k per annum (baseline: 19.71%; analytic: 17.72%), and about one quarter of families were living in areas of the highest deprivation according to IMD (baseline: 19.98%; analytic: 28.43%). Finally, infants in the analysis sample were born with lower birthweight z-scores (baseline: -0.87, analytic: -1.23) and showed a higher prevalence of RIWG to 12-months of age (baseline: 66.41%, analytic: 79.03%)

Table 3.2. Characteristics of the Individual Gemini Twins included in Adjusted LMM Sample Compared to Baseline Sample

		Baseline Sample of Gemini Infants (n=4804)		Adjusted LMM Sample of Gemini Infants (n=1998)*	
		N	Mean (SD) or %	*N*	Mean (SD) or %
Infant Feeding Exposures					
Infant Feeding Modality[a] (3-groups)					
	Exclusively Breastfed	654	14.90	317	15.87
	Mixed Fed	2,100	47.86	967	48.40
	Formula Fed	1,634	37.24	714	35.74
Infant Feeding Modality[b] (7-groups)					
	Exclusively Breastfed	543	12.37	297	14.86
	Breastfed and Expressed Fed	151	3.44	57	2.85
	Expressed Fed	207	4.72	39	1.95
	Breastfed and Formula Fed	1,201	27.37	674	33.73
	Breast, Formula Fed and Expressed Fed	259	5.90	120	6.00
	Formula Fed and Expressed Fed	393	8.96	97	4.85
	Formula Fed	1,634	37.24	714	35.74
Infant Characteristics					
Zygosity					
	Dizygotic	3,232	68.33	1,414	70.77
	Monozygotic	3,232	31.67	584	29.23
Sex					
	Boy	2,386	49.67	972	48.65

		2,418	50.33	1,026	51.35
	Girl				
Gestational Age (in weeks)		4,784	36.20 (2.48)	1,998	37.43 (1.05)
Birthweight z-score		4,639	-.87 (0.96)**	1,998	-1.23 (0.87)**
Change in Weight-for-age z-score 0-3 months		4,232	.49 (1.13)*	1,984	.77 (1.01)*
Change in Weight-for-age z-score 0-12 months		2,337	1.16 (1.19)**	1,116	1.49 (1.03)**
Rapid Growth at 3-months[c] (z-score >0.67)					
	No Rapid Weight Gain	2,334	55.15	890	44.86
	Rapid Weight Gain	1,898	44.85	1,094	55.14
Rapid Growth at 12-months[c] (z-score >0.67)					
	No Rapid Weight Gain	785	33.59**	234	20.97**
	Rapid Weight Gain	1,552	66.41	882	79.03
Mode of Delivery					
	Caesarean	3,118	65.20	1,303	65.22
	Vaginal	1,664	34.80	695	34.78
Days in Specialist Care					
	0 days	3,622	76.32	1,611	80.63
	1 – 4 days	593	12.49	281	14.06
	5 – 9 days	212	4.47	80	4.00
	10 + days	319	6.72	26	1.30
Introduction of Solid Foods Timing					
	≥6 months	1,105	24.37	414	20.72
	5 to< 6 months	1,444	31.85	687	34.38
	4 to <5 months	1,385	30.55	661	33.08
	<4 months	600	13.23	236	11.81
Family Characteristics					
Age at Delivery (in years)		4,792	32.94 (5.18)	1,998	33.33 (4.93)
Maternal BMI		4,676	25.10 (4.75)	1,998	24.94 (4.72)
Maternal Ethnicity					
	White	4,462	92.96	1,890	94.59
	Other	338	7.04	108	5.41
Maternal Marital Status					

	Married	4,552	94.83	1,913	95.75
	Divorced or Separated	62	1.29	18	0.90
	Single	186	3.88	67	3.35
Maternal Smoking During Pregnancy					
	Yes	536	11.17	192	9.61
	No	4,264	88.83	1,042	90.39
Parity					
	None	2,398	51.42	1,042	52.15
	One or More	2,266	48.58	956	47.85
Gestational Diabetes					
	Yes	140	3.09	55	2.75
	No	4,392	96.91	1,943	97.25
Socioeconomic Position					
National Statistics Socioeconomic Class (NS-SEC)[d]					
	High	2,662	61.84	1,360	68.07
	Middle	744	17.28	319	15.97
	Lower	899	20.88	319	15.97
Maternal Educational Attainment					
	Higher Degree	1,844	54.20	1,154	57.76
	A level / GSCE or Equivalent	1,376	40.45	775	38.79
	No Qualification	182	5.35	69	3.45
Home Ownership Status					
	Own without mortgage	254	7.47	150	7.51
	Own with mortgage	2,484	73.02	1,537	76.93
	Rent privately	388	11.41	193	9.66
	Rent from local authority	240	7.05	118	5.91
Gross annual household income[e]					
	<22.5k	646	19.71	354	17.72
	22.5k to 37.5k	838	25.56	493	24.67
	37.5k to 52.5k	664	20.26	391	19.57
	52.5k to 67.5k	418	12.75	296	14.81
	>67.5k	712	21.72	464	23.22
Index of Multiple Deprivation[f]					

	Least Deprived	950	19.98***	206	10.31***	
	Not Deprived	952	20.23	313	15.67	a
	Mid	950	19.46	401	20.07	
	Deprived	952	20.03	510	25.53	
	Most Deprived	950	19.98	568	28.43	
Number of Bedrooms		4,800	3.26 (0.96)	1,998	3.28 (0.93)	
Number of Cars		4,800	1.60 (0.72)	1,998	1.61 (0.68)	
Composite SES Measure		4,526	4.33 (1.36))	1,998	4.47 (1.35)	

*** p < 0.001; ** p < 0.01; * p < 0.05.

*Inclusive of Both LMMs using 3-groups Infant Feeding Method Variable and 7-group Infant Feeding Modality Variable

[a]Exclusively Breastfed: Fed breast milk from the breast or from expressed milk; Expressed Milk Fed; Fed with primarily expressed milk from a bottle or equal amount of breastmilk from the breast and expressed milk from a bottle; Mixed-Fed: Fed with a combination of breast milk from the breast, expressed milk in a bottle, and formula milk; Formula Fed: Fed with formula milk

[b]Exclusively Breastfed: Fed exclusively breast milk from the breast; Breastfed and Expressed Fed: Fed with a combination of breast milk from the breast and expressed milk in a bottle; Expressed Fed: Fed with expressed milk in a bottle; Breastfed and Formula fed; Fed with a combination of breast milk from the breast and formula milk; Breast, Formula and Expressed: Fed with a combination of breast milk from the breast, expressed milk in a bottle, and formula milk; Formula and Expressed Fed: Fed with a combination of expressed milk from a bottle and formula milk; Formula Fed: Fed with formula milk

[c]Change in weight for age z-score >0.67 indicative of rapid weight gain

[d] Household NS-SEC based on the Higher Ranking Position of the two full NS-SECs

[e]Annual household income was assessed with the following question 'What is the total household income (before tax deduction)?'.

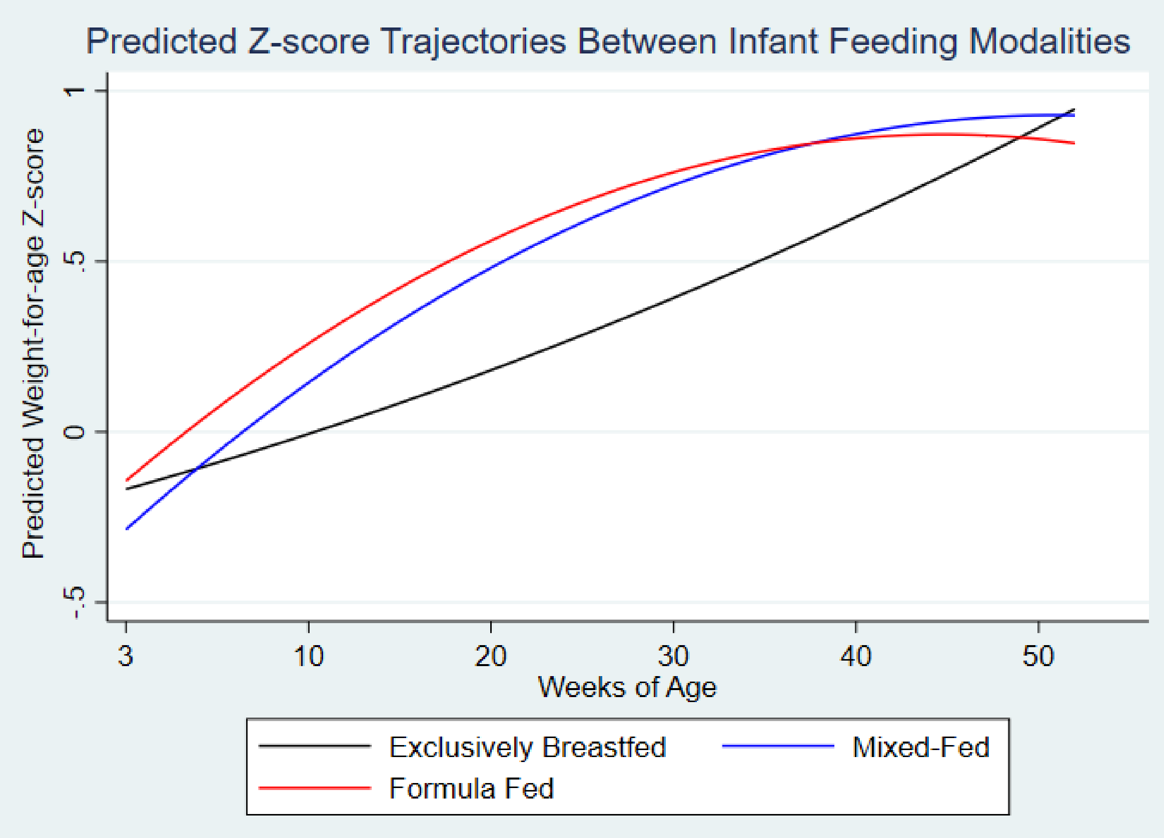
[f]5 quintiles of deprivation, 1 = 'score ≤8.49 (least deprived quintile)', 2 = '8.5–13.79', 3 = '13.8–21.35', 4 = '21.36–34.17', 5 = '≥ 34.18 (most deprived quintile)'

% for analytic sample do not include missing data to represent distribution of variables across infants included in the sample

3.4.2.2 RQ 1: Do weight gain trajectories to 12-months of age vary across a broad 3-group measure of Infant Feeding Modality; breastfed, mixed-fed and formula fed infants?

Figure 3.4 summarises the predicted weight-for-age z-score trajectories across the broad 3--level infant feeding modality for term Gemini twins (n=1998). As compared to breastfed infants, infants fed with mixed-feeding (β = -0.11; 95% CI: -0.25, 0.17) or formula feeding (β = 0.02; 95% CI: -0.12, 0.17) did not show varied z-scores at baseline (mean = 3.12 weeks of age). When considering both the IFMxage (linear increase) and IFMxage^2 (quadratic increase) effects, 'mixed-fed' infants began at the lowest z-score and showed the most steep increases in z-score to 35-weeks of age (~9 months) (IFMxtime; β = 0.03 z-score per week; 95% CI: 0.02, 0.05) followed by a plateau from 35 to 52 weeks of age (IFMxtime^2; β = -0.00059; 95% CI: -0.00086, -0.00032). Formula fed infants showed a similar pattern to mixed-fed infants up to 35-weeks of age, showing steeper increases in z-score than breasted infants (IFMxtime; β = 0.03; 95% CI: 0.02, 0.04), followed by a more pronounced plateau of z-score increase from 35- to 52- weeks of age (IFMxtime^2; β = -0.00063; 95% CI: -0.00091, -0.00035). These trajectories are presented in Figure 3.6. The table of estimates produced by the unadjusted model are provided in Appendix II, and the unadjusted model in Appendix I. The adjusted model was a better fit to the data (AIC = 10221.82 BIC = 10495.82) than the unadjusted model (AIC = 47924.94, BIC = 47924.14)

Figure 3.4. Infant Feeding Modalities (3-groups) and Predicted Weight for Age Z-score Trajectories to 12-months of age (n=1998)



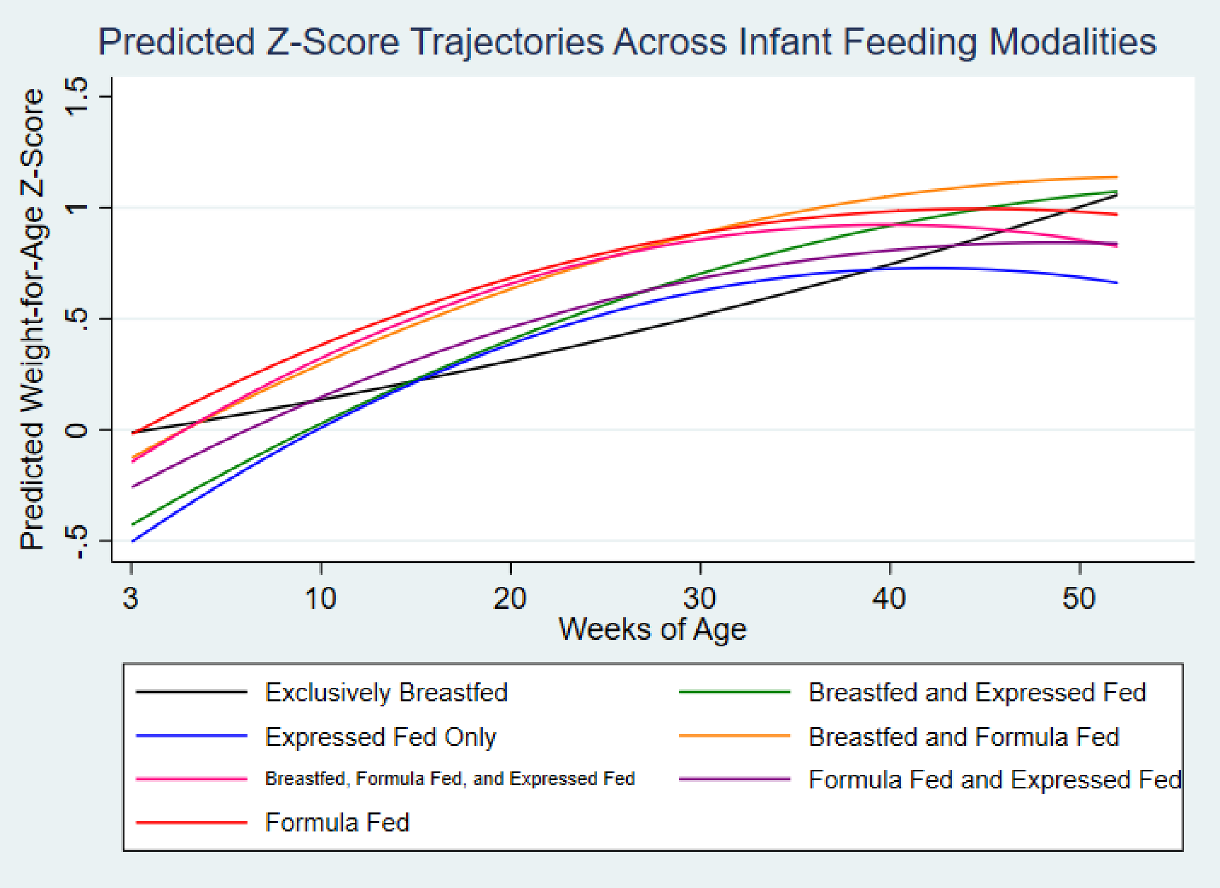
Weight for age Z-score; Calculated using UK-WHO growth refence data adjusting for age, sex, and gestational age for each weight measurement.
Adjusted for birthweight, zygosity, sex, gestational age, days spent in specialist care, maternal age at delivery, mode of delivery, maternal BMI, maternal ethnicity, maternal marital status, maternal smoking during pregnancy, gestational diabetes, composite socioeconomic position, introduction of solid foods, and clustering within families
Sample sizes: Exclusively Breastfed (n= 317): Fed breast milk from the breast or from expressed milk; Mixed-Fed (n=967): Fed with a combination of breast milk from the breast, expressed milk in a bottle, and formula milk; Formula Fed (n= 714): Fed with formula milk

3.4.2.3 RQ 2: Do weight gain trajectories to 12-months of age vary across the full range of feeding modalities?

Figure 3.5 summarises the predicted weight-for-age z-score trajectories across IFMs for eligible twins (n=1998). Overall, the varied combinations of mixed feeding were associated with varying weight z-score trajectories across the first year of life. Infants fed with expressed milk either exclusively or in combination, 'expressed fed' (β = -0.49; 95% CI: -0.83, -0.14), 'breastfed and expressed fed' (β = -0.41; 95% CI: -0.68, -0.15) and 'formula and expressed fed' (β = -0.24; 95% CI: -0.47, 0.02), showed significantly lower z-scores at baseline (mean = 3.12 weeks of age) as compared to the exclusively breastfed infants.

When evaluating the predicted rate of increase in z-scores to 12-months of age, each mixed-fed or formula-fed IFM group showed significantly steeper increases in z-scores over time as compared to exclusively breastfed infants. When considering both the IFMxage (linear increase) and IFMxage^2 (quadratic increase) effects, 'breastfed and formula fed' infants showed the most consistently steep increases in z-score to 12-months of age (IFMxtime; β = 0.03; 95% CI: 0.03, 0.05; IFMxtime^2; β = -0.00056; 95% CI: -0.00085, -0.00027), followed by primarily formula fed infants (IFMxtime; β = 0.04; 95% CI: 0.02, 0.05; IFMxtime^2; β = -0.00064; 95% CI: -0.00092, -0.00035) and 'breast, expressed fed, and formula fed' infants (β = 0.04; 95% CI: 0.02, 0.06; IFMxtime^2; β = -0.00080; 95% CI: -0.00120, -0.00040). However, these two later groups showed a stronger plateauing in their z-score trajectories from 30 weeks onwards, as presented in **Figure 3.5**. 'Expressed fed and formula' (β = 0.03; 95% CI: 0.00, 0.05; IFMxtime^2; β = -0.00060; 95% CI: -0.00102, -0.00018) and 'expressed fed infants' (β = 0.04; 95% CI: 0.01, 0.07; IFMxtime^2; β = -0.00082; 95% CI: -0.00141, -0.00024) showed similar weight gain trajectories, with steeper increases in weight to 30 weeks of age, followed by a plateau from 30 to 52 weeks of age. The table of estimates produced by the unadjusted model are provided in Appendix I, and the adjusted model in Appendix II. The adjusted model was a better fit to the data (AIC = 35207.95. BIC = 35578.32) than the unadjusted model (AIC =47914.49, BIC = 48013.78).

Figure 3.5. Infant Feeding Modalities and Predicted Weight for Age Z-score Trajectories to 12-months of age (n=1998)



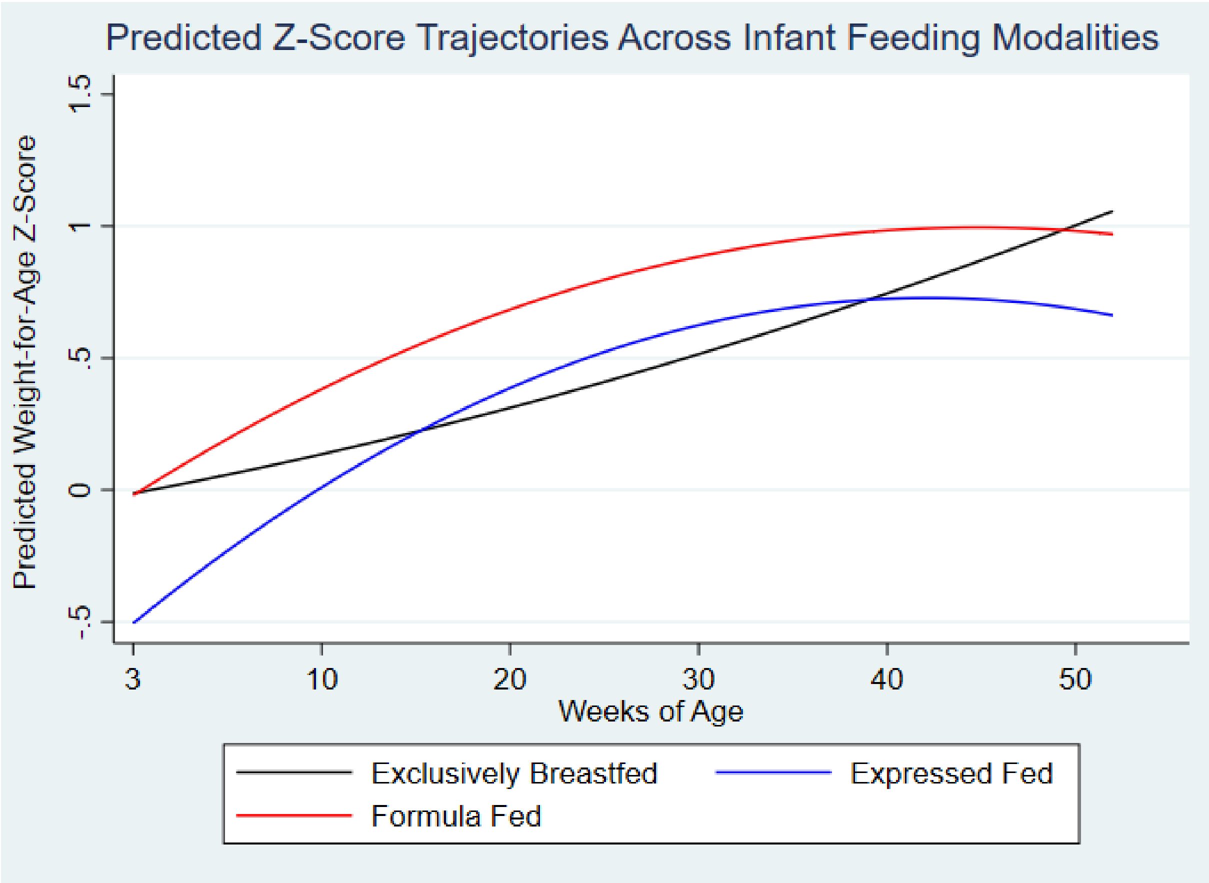
Weight for age Z-score; Calculated using UK-WHO growth refence data adjusting for age, sex, and gestational age for each weight measurement.
Adjusted for birthweight, zygosity, sex, gestational age, days spent in specialist care, maternal age at delivery, mode of delivery, maternal BMI, maternal ethnicity, maternal marital status, maternal smoking during pregnancy, gestational diabetes, composite socioeconomic position, introduction of solid foods, and clustering within families
Sample sizes: Exclusively Breastfed: Fed exclusively breast milk from the breast (n=297); Breastfed and Expressed Fed (n=57): Fed with a combination of breast milk from the breast and expressed milk in a bottle; Expressed Fed (n=39): Fed with expressed milk in a bottle; Breastfed and Formula fed (n=674); Fed with a combination of breast milk from the breast and formula milk; Breast, Formula and Expressed (n=120): Fed with a combination of breast milk from the breast, expressed milk in a bottle, and formula milk; Formula and Expressed Fed (n=97); Fed with a combination of expressed milk from a bottle and formula milk; Formula Fed (n=714); Fed with formula milk

3.4.3 Aim 3.2: Explore mechanisms that may promote rapid weight gain in formula fed infants; the bottle versus formula milk

3.4.3.1 RQ 3: Do weight gain trajectories to 12-months of age vary between i) breastfed infants ii) expressed infants and iii) formula fed infants?

Figure 3.6 presents the weight gain trajectories of the three feeding modality groups; i) infants fed with exclusively breastmilk from the breast ii) infants fed with predominantly breastmilk expressed into a bottle and iii) infants fed with formula milk. Expressed fed infants began at a lower z-score (β = -0.49; 95% CI: -0.83, -0.14) than infants fed directly from the breast but showed steeper increases in their trajectories over time (IFMxtime; β = 0.04; 95% CI: 0.01, 0.07; IFMxtime^2; β = -0.00082; 95% CI: -.00141, -.00024). Hence, when examining weight gain trajectories, the pattern of growth in infants fed expressed breastmilk is more similar to infants fed from exclusively formula milk, than EBF infants. However, expressed fed infants carry a lower weight at baseline and throughout the first year of life. The table of estimates produced by the unadjusted model are provided in Appendix I, and the crude adjusted model in Appendix II. The adjusted model was a better fit to the data (AIC = 35207.95. BIC = 35578.32) than the unadjusted model (AIC =47914.49, BIC = 48013.78).

Figure 3.6. Predicted Weight for Age Z-score Trajectories to 12-months of age between Exclusively Breastfed Infants, Expressed Fed Infants, and Formula Fed Infants (n=1050)



Weight for age Z-score; Calculated using UK-WHO growth refence data adjusting for age, sex, and gestational age for each weight measurement.
[a] Adjusted for birthweight, zygosity, sex, gestational age, days spent in specialist care, maternal age at delivery, mode of delivery, maternal BMI, maternal ethnicity, maternal marital status, maternal smoking during pregnancy, gestational diabetes, composite socioeconomic position, introduction of solid foods, and clustering within families
[c]Exclusively Breastfed (n=297): Fed exclusively breast milk from the breast; Expressed Fed (n=39): Fed with expressed milk in a bottle; Formula Fed (n=714); Fed with formula milk

3.4.4 Aim 3.3: Apply the Discordant Twin (DT) design to account for social confounding from associations between IFMs and infant weight gain trajectories across the first year of life

3.4.4.1 Sample Characteristics

Table 3.3 presents the characteristics of all MZ and DZ twin pairs from the baseline sample of Gemini, as compared to the discordant twin sample included in the DT analyses (n=108 twin pairs). A greater proportion of the twin pairs who were discordant on IFMs feeding were dizygotic, compared to non-discordant twin pairs (discordant; n=91; 84.26%, baseline sample; n=1616, 68.33%). Moreover, twin pairs discordant for IFMs were more likely to differ in sex than non-discordant twin pairs (discordant sample: n=60; 54.55%; baseline sample: n=816; 33.97%). Finally, as compared to baseline twin pairs discordant twin were more likely to spend 1-4 days in specialist care (discordant; n=22, 20.00%, baseline sample: n=287, 12.06%). Twin pairs discordant for IFMs has smaller birthweight z-scores at birth than the baseline sample of twin pairs (discordant sample: n=108; -1.32; baseline sample: n=2314; -0.94).Twin pairs discordant did not vary from non-discordant twins on mode of delivery, timing of introduction to solid foods, ethnicity, NS-SEC, or index of multiple deprivation.

Table 3.3. Characteristics of Gemini Twin Pairs at Baseline and Gemini Twins Pairs Discordant on Infant Feeding Modality Included in the Discordant Twin Analysis

		Gemini Twin-Pairs (n=2404)		Twin Pairs Discordant in Infant Feeding Modalities[a] (n=108)	
Characteristics		*N*	*% or M (± SD)*	*N*	*% or M (± SD)*
Mean Birthweight Z-score		2,314	-0.94 (0.87)*	108	-1.32 (0.72)*
Zygosity					
	Dizygotic	1,616	68.33*	91	84.26*
	Monozygotic	749	31.67	17	15.74
Gestational Age (in weeks)		2,392	36.20 (2.48)	108	37.38 (0.94)
Mode of Delivery					
	Caesarean	1,559	65.20	82	74.55
	Vaginal	832	34.80	26	25.45
Timing of introduction to solid foods					
	≥6 months	562	24.78	21	19.09
	5-6 months	721	31.79	38	34.55
	4-5	684	30.16	39	35.45
	<4 months	301	13.27	12	10.91
Sex					
	Male, Male	785	32.68**	18	17.27**
	Female Female	801	33.35	30	28.18
	Male, Female	816	33.97	60	54.55
Ethnicity					
	White	2,231	92.96	104	96.36
	Other	169	7.04	4	3.64
Days in Specialist Care					
	0 days	1,836	77.14*	79	71.82*
	1 – 4 days	287	12.06	22	20.00
	5 – 9 days	103	4.33	8	7.27

	10 + days	154	6.47	1	0.91
Parity					
	None	1,199	51.42	69	65.09
	One or More	1,133	48.58	38	34.91
National Statistics Socioeconomic Class (NS-SEC)[b]					
	High	1,515	63.28	73	66.97
	Middle	470	17.00	17	15.60
	Lower	472	19.72	19	17.43
Index of Multiple Deprivation[c]					
	Least Deprived	613	25.78	17	15.89
	Not Deprived	573	24.10	21	19.63
	Mid	476	20.02	28	26.17
	Deprived	412	17.33	26	24.30
	Most Deprived	304	12.78	16	14.02

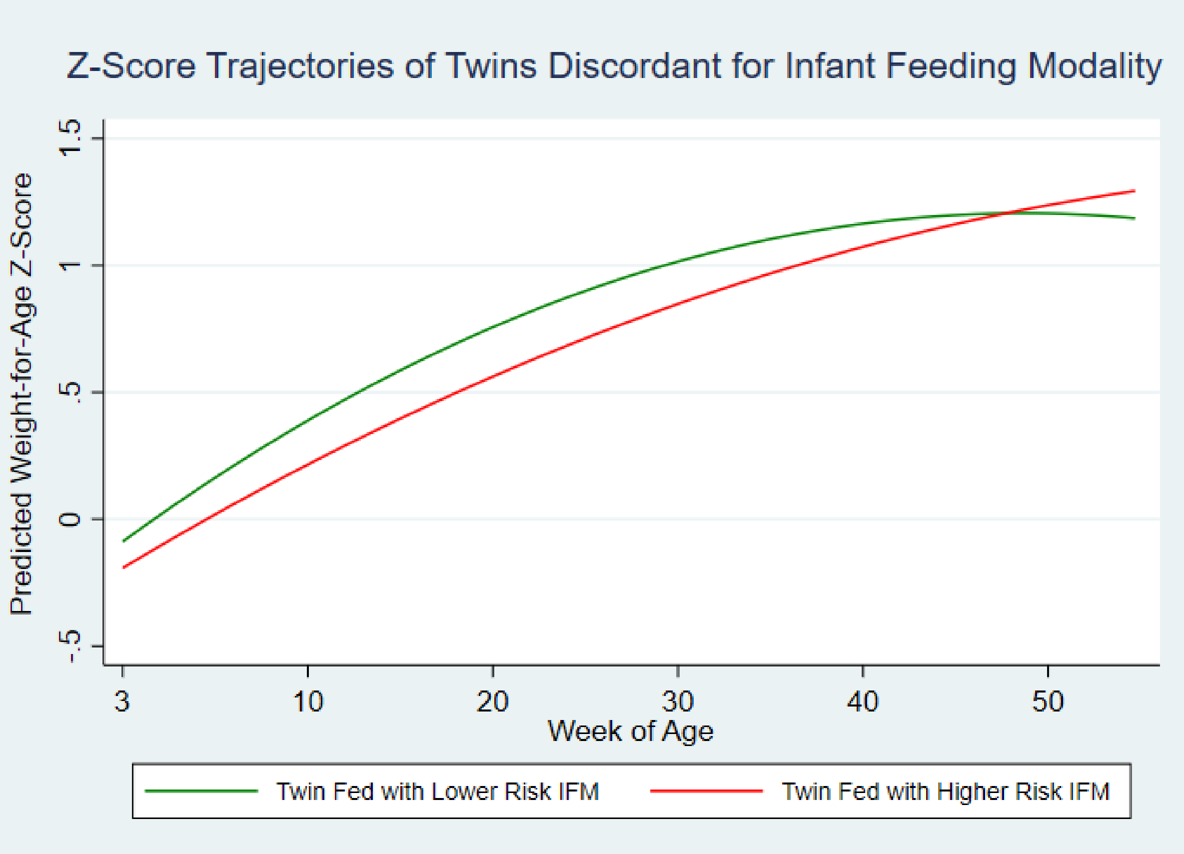
*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.

[a] Twin Discordant in Infant Feeding Modality such that one twin was fed with more formula milk or expressed breastmilk and one twin was fed was given less formula or expressed breastmilk than their co-twin
[b]Household NS-SEC based on the Higher Ranking Position of the two full NS-SECs
[c]5 quintiles of deprivation, 1 = 'score ≤8.49 (least deprived quintile)', 2 = '8.5–13.79', 3 = '13.8–21.35', 4 = '21.36–34.17', 5 = '≥ 34.18 (most deprived quintile)'
% for analytic sample do not include missing data to represent distribution of variables across infants included in the sample

3.4.4.2 RQ 4: Do twin pairs discordant for IFMs show varying weight gain trajectories to 12-months of age?

DT analysis compared z-score trajectories between co-twins discordant on infant feeding such that one twin was fed with a higher risk feeding modality than their co-twin (n=108 twin pairs). There was no statistically significant difference in z-score at baseline (β = -0.00 95% CI: -0.03, 0.02) or in rate of z-score increase to 12-months (β = -0.01; 95% CI: -0.29, 0.08) between the twin fed with a higher risk feeding modality than their co-twin fed with a lower risk feeding modality (ref). These results are presented in **Figure 3.7**. The table of estimates produced by the DT model are provided in Appendix III. The adjusted model was a better fit to the data (AIC = 668.89 BIC = 748.61) than the unadjusted model (AIC = 678.33 BIC = 740.28)

Figure 3.7. Weight for Age Z-Score Trajectories to 12 months Between Twin Pairs Discordant for Infant Feeding Modality (n=108 Twin Pairs)



Twin Pairs Discordant in Infant Feeding Modality; one twin was fed with higher risk IFM (more formula milk or expressed breastmilk) and one twin was fed was lower risk IFM (given less formula or expressed breastmilk than their co-twin)

Adjusted for; Difference in sex, difference in birthweight z-score, difference in days spent in specialist care, difference in timing of introduction to solid foods

3.5 Discussion

3.5.1 Summary of Findings

Chapter three sought to explore relationships between a detailed measure of infant feeding modalities (IFMs) and rapid weight gain trajectories across the first year of life. No previous studies have been able to examine these relationships using as detailed a measure of IFM as the one derived (7-groups). In addition. This study was able to go beyond cross-sectional observations by considering the association of the IFMs with longitudinal trajectories of infant growth.

The chapter first aimed to model variations in weight gain trajectories between a broad (3-group) measure of IFMs and detailed (7-group) measure of IFMs. Looking at weight gain trajectories across broad IFMs, was apparent that both predominantly formula fed infants and mixed-fed (infants fed with a mixture of formula and breastmilk showed a steeper acceleration in weight for age z-scores across the first year of life when compared to EBF infants. EBF showed a more linear increases in weight across the period, whilst both mixed and formula fed infants showed more quadratic patterns, with steeper increases to 35 weeks followed by a plateauing or decrease in z-scores from 35 to 52 weeks. This suggests formula milk may increase risk of RIWG not only when it is introduced exclusively but also when introduced alongside breastfeeding.

For the second research question, I examined weight gain trajectories between a more detailed 7-group measure of IFMs, covering varied combinations of breastfeeding, expressed breastfeeding and formula feeding. Overall, there was no stepwise increase in the rate of weight gain as the proportion of formula milk increased across IFMs. Together, this suggests that there may be a more complex and nuanced relationship between IFMs and infant growth than previously assumed and reported by the literature.

Finally, it is of note that the z-scores differed meaningfully across IFMs at baseline (~3-weeks of age). Specifically, EBF and formula fed infants showed the higher z-scores at baseline, as compared to the other 5 mixed feeding groups. This finding contradicts the assumed causal influence of formula feeding on rapid growth – as breastfed infants should have lower z-scores than infants supplemented with formula milk at all timepoints, including baseline which was ~3 weeks of age in the current study. These finding therefore points towards potential reciprocity in infant feeding decisions - such that slower infant growth in the early weeks may directly influence IFMs adopted by caregivers. For the second aim of the chapter, expressed fed infants, or those fed primarily with breastmilk from a bottle in the first 3-months of life, showed steeper increases in weight gain than infants fed exclusively from the breast. Moreover, expressed fed patterns of weight gain were more similar to those of infants fed predominantly with formula milk. This comparison of feeding modality (breast vs. bottle) thereby implicates bottle-feeding and its associated mechanisms in rapid weight development.

For the final aim, the genetically sensitive DT design explored whether discordance in IFMs across twins leads to varied weight gain trajectories, removing residual confounding from shared environmental exposures. Overall, twins fed with a higher risk feeding modality, characterised by more bottle or formula feeding, did not show steeper weight gain than their co-twin. However, upon visual inspection of the weight gain trajectories it was apparent, although not statistically significant, that the twin fed with more bottle or formula feeding showed a lower z-score at baseline to ~40 weeks of age (=9 month). After ~40 weeks, this twin then *caught up* to their co-twin and thereafter surpassed them in weight z-score. This trend may indicate that non-shared differences between twins may also confound the IFMs to infant growth association. In other words, there may exist a bi-directional relationship such that formula feeding might be introduced to one twin who is growing slower, to help enable them to catch up to their co-twin. Therefore, whilst the DT comparisons removed confounding from shared environmental exposures, confounding from non-shared characteristics need to be explored further.

3.5.2 Discussion of Findings Compared to Previous Literature

3.5.2.1 Aim 3.1: Explore variations in infant weight gain trajectories across the first year of life between a broad (3-group) and detailed measure of Infant Feeding Modalities (7-group)

The first aim of the current chapter was to explore whether weight gain trajectories to 12-months of age varied across IFMs; using both a broad 3-group measure a detailed 7-group measure of IFM. A key limitation of previous studies on this topic is the predominant use of cross-sectional measures of RIWG[53,54] – such as change z-scores from birth to 6- or 12-months of age. This can largely be ascribed to the rarity of repeated anthropometric data across infancy amongst birth cohorts. This study therefore adds to the literature by modelling growth trajectories, utilising the rich set of anthropometric data in Gemini. It was apparent that both formula fed infants and infants fed with a mixture of formula and breastmilk showed a steeper acceleration in weight trajectories as compared to exclusively breastfed infants. This finding aligns with other birth cohorts who have been able to model weight trajectories, such as the Generation R Study (n=5047) where infants who were exclusively formula fed (B=0.24, 95%CI: 0.17, 0.32) or partially formula fed (B=0.23, 95%CI: 0.18, 0.27) until 4 months showed higher increase in weight z-scores relative to EBF infants to 12-months.[53] Together, this suggests that mixed-fed infants, which are the largest IFM group in the UK (55% of mothers report mixed-feeding by 6-weeks of age)[142], show an observationally similar risk of RIWG when as exclusively formula fed infants. Therefore, infants fed with a combination of breast and formula milk should be targeted by intervention or policy efforts seeking to support healthy growth.

However, the key aim of the present chapter was to investigate how weight gain trajectories varied across a more granular 7-group measure of IFMs. This granular measure was made possible due to the detailed feeding data provided by Gemini mothers in infancy – also a rarity in birth cohorts. Literature regarding IFMs and infant growth is therefore often limited by poor operationalization of IFMs[51]. Firstly, 'breastfed' groups of infants often represent variable proportions of actual breastfeeding such that it may contain either exclusive breastfeeding from the breast or 'any' provision of breastmilk. Moreover, 'mixed fed' infants are often combined into one group although they may contain an array of differing combinations of breastfeeding, expressed breastfeeding and formula feeding. The 7-group infant feeding modality therefore sought to disentangle both 'breastfeeding' and 'mixed-feeding' to undertake one of the most detailed investigations of weight gain trajectories to date. Overall, each mixed-or-formula fed group showed significantly steeper increases in weight over time as compared to exclusively breastfed infants. Moreover, when examining these weight gain trajectories, there was no evidence of a clear stepwise increase in speed of weight gain as the degree of formula feeding increased across mixed-feeding groups. For example, infants fed with predominantly expressed breastmilk showed similar weight gain trajectories to those fed with expressed breastmilk and formula milk. Moreover, infants fed with a combination of breastmilk from the breast and expressed breastmilk showed similarly steep weight gain than infants fed with a combination of formula milk and expressed breastmilk. These two observations, contradict the commonly presumed *causal* pathway from *more* formula feeding to *more* RIWG.[41] However, it is also important to reflect on the varying z-scores at baseline (~3-weeks of age) across IFMs. Both exclusively breastfed and exclusively formula fed infants were the largest at baseline, with the varied combinations of mixed-feeding showed lower z-scores. One explanation for this finding, is that the infants may weaned away from exclusive breastfeeding in response to their slower growth patterns in the early weeks of life. Therefore, exclusively breastfed and formula fed infants showed higher weight at ~3-weeks, as they were growing sufficiently and therefore caregivers did not modify their feeding methods. These findings point towards potential reciprocity in infant feeding decisions - such that infant growth in the early weeks may *precede* IFMs.[40] These bi-directional effects, or reciprocity, were explored further in Chapter Four, and therefore relevant literature will be discussed there.

3.5.2.2 Aim 3.2: Explore mechanisms that may promote rapid weight gain in formula fed infants; the bottle versus formula milk

The present study also aimed to disentangle potential mechanisms which might place infants fed with formula milk at risk for RIWG. Although many observational studies have demonstrated a higher risk for RIWG amongst formula fed infants[19], numerous mechanisms remain under investigation. On one hand, it has been proposed that formula milk may lead to RIWG, as the varied nutritional makeup may be related to changes in hormones which stimulate accelerated weight gain or adiposity deposition.[30,109,143] On the other hand, it has also been proposed that behavioural differences in feeding that occur when feeding through a bottle versus from the breast, may encourage infants to drink more rapidly and/or enable less responsive feeding practices.[54,144] For instance, breastfed infants need to actively suckle, and therefore feeding through a bottle may enable infants may be more passive in feeding. This in turn might enable caregivers to garner more control over feeding and undermine infants' self-regulation of appetite more easily in the long term.[108] Hence, the question remains; why do formula fed infants show more RIWG – is it the bottle or the milk? One useful way to answer this question is to compare infants fed breastmilk but from differing modalities; a breast and a bottle. If infants fed with breastmilk expressed into a bottle show more rapid growth than those from fed only from the breast, this would suggest that bottle-feeding and associated mechanisms might contribute to RIWG. Few cohorts and observational studies to date contain sufficiently detailed measures of breastfeeding to disentangle these two feeding modalities. However, one previous study undertook such comparisons in an American sample. In the Infant Feeding Practices Study II (n=1899)[50], a small group of infants fed with exclusively expressed breastmilk from a bottle gained more weight per month (n=34, weight gain/m = 88.83, 95%CI: 13.19, 164.47) as compared to breastfed infants exclusively fed from the breast (n=333, ref (not reported)), controlling for numerous socio-demographic factors and infant characteristics.[145] Similar to Li and colleagues' findings, our results within Gemini show that a group of expressed fed infants (n=39) also showed steeper increases in weight gain to one-year as compared to exclusively breastfed infants fed from the breast. Expressed fed infants therefore showed more similar patterns of weight gain to formula fed infants than breastfed from the breast infants. These findings suggest that bottle-feeding, and its associated behaviours, may be a potential mechanism underlying accelerated weight gain in infancy. However, it is important to note that Gemini did not have a large enough sample of infants fed with exclusively expressed breastmilk to ~3-months of age (n=14), and therefore infants fed *predominantly* breastmilk from a bottle, where mothers reported feeding with mostly expressed breastmilk and some formula milk across the first 3-months of age, was used. Hence, the small proportion of formula given to these infants may have influenced their weight gain and increased similarity to those who were formula fed. Nonetheless, this proportion of formula feeding was small, and previous findings in exclusively expressed fed infants confer our results.[50] Second, it is important to consider how the small (n=39) group of infants primarily fed with expressed breastmilk to ~3-months of age, may have been highly unique and fed in this manner due to health complications or experiences which could influence growth trajectories and should be considered when interpreting these results. Despite these limitations, the uniquely detailed feeding modality data paired with repeated growth data offered a unique opportunity to disentangle bottle-feeding from formula feeding in Gemini. Whilst these findings would benefit from replication in other samples, they carry numerous implications which will be discussed in section 3.5.2.4.

3.5.2.3 Aim 3.3: Apply the Discordant Twin (DT) design to remove social confounding from associations between IFMs and infant weight gain trajectories across the first year of life

For the final aim of this chapter, I applied DT design to examine weight gain trajectories between IFMs, removing residual confounding from social and economic circumstances. As discussed in the literature review (See section 12.2.2) a persistent limitation of observational designs measuring associations between breastfeeding and weight development is confounding from the social gradient of breastfeeding.[112] As breastfeeding is strongly socially graded in the UK[43,112], the benefits of breastfeeding on infant weight gain may represent a proxy for other benefits provided by socio-economic advantage. This might include the ability to afford a higher quality diet during complementary feeding or the use of different parental feeding practices. The “gold-standard” method of removing such confounding from socioeconomic advantage would be a randomised control trial (RCT) design randomising infants to receive only breastmilk versus formula milk. As these designs would be unethical, given the benefits of breastfeeding outside of weight development, only breastfeeding promotion trials have been able to measure the influence of enhanced breastfeeding rates on infant growth. Whilst such studies, such as the PROBIT trial[39], have not demonstrated the benefits of breastfeeding promotion on infant growth outcomes, it remains challenging to know whether these null effects were the result of removing social confounding, or simply the “spill over” of formula feeding within the breastfeeding promotion intervention group. Therefore, another research design particularly useful in seeking to remove confounding from shared environmental exposures is sibling designs and twin studies. Specifically, the DT design can remove residual confounding from the shared home and social environment as these are completely shared across the twin pair. When comparing trajectories twins discordant for IFMs in this study, we did not observe significantly steeper weight gain trajectories in the twin fed with more formula or bottle feeding than their co-twin. These results largely mirror the scarce number of previous sibling studies on this topic. For instance, a recent review of sibling studies on breastfeeding and the risk of overweight from age 2 highlighted that only 1 of 4 sibling analyses identified significant associations between breastfeeding and childhood weight outcomes under sibling comparisons.[45] Hence, the benefits of breastfeeding on infant weight development that are observed in unrelated individuals may reflect, at least partly, socioeconomic advantages (e.g. healthier diets and lifestyles in later infancy and early childhood). However, whilst the DT design is able to remove confounding from shared environmental exposure, it does not remove confounding from non-shared exposures – such as unique infant weight gain or random illness – which may influence both infant feeding exposures and infant growth outcomes.[35] Hence, when twins are fed differently, which is rare, it is therefore likely to be a meaningful response towards differences between co-twins. As discordance in feeding is rare, these non-shared characteristics are likely to be very influential within the niche subset of co-twins fed differently in infancy. In this way, the association between IFMs and infant growth may be less observable amongst such twins, not only due to the removal of social confounding, but also due to important non-shared differences between the twins that can influence the relationship between IFMs and Infant weight gain. Hence, our findings cannot conclusively determine that the lack of association between IFMs and infant growth under the DT comparisons was entirely the result of removing social confounding, as non-shared characteristics are likely to influence this relationship.

In line with this, upon inspection of the DT weight gain trajectories in Gemini, co-twins fed with more bottle- or formula feeding appeared to be smaller in early infancy – such that they had a lower weight z-score to ~40 weeks of age. Then, after ~40 weeks, this twin caught up with to their co-twin and thereafter surpassed them. I postulate that this finding may point towards important bi-directional influences in feeding - such that that the introduction of more formula or bottle feeding may be a response to slower growth in earlier infancy. In other words, where one twin is growing slower than their co-twin, formula feeding might be introduced to help them *catch up* to their sibling. Whilst no twin or sibling studies prior to ours have been applied to IFMs and infant growth, analyses from trials such as the CBGS have begun to highlight the presence of reciprocity towards slower growth in early infancy[70] – these will be discussed in the next chapter. Moreover, studies from later childhood have highlighted parent-child reciprocity in feeding, such that parents adapt their parental feeding practices in line with the expressed appetite and weight development of their children.[71,72,78,146] These studies are discussed under the next chapter focusing on parent-child reciprocity in infant feeding.

3.5.3 Future Directions and Implications

First, the current chapter observed more rapid growth to 12-months of age amongst both formula-fed infants and those fed with a mixture of formula and breastmilk. This finding suggests that the observational risk of RIWG is not limited to infants fed exclusively with formula milk. Therefore interventions and policies seeking to support healthy growth or modify infant feeding practices or behaviours should consider and include 'mixed feeders'.

Second, breastfed infants fed through a bottle showed steeper increases in weight gain to 12-months of age, as compared to infants fed exclusively from the breast. This finding implicates bottle-feeding in RIWG and suggests that bottle-feeding behaviours may be a promising target for interventions seeking to support healthy growth in infancy.
This is an important finding as feeding interventions seeking to promote healthy growth and infant development largely focus on breastfeeding promotion, which have been largely "unsuccessful" given the high prevalence of formula feeding in the UK. An alternative approach may therefore be to promote appropriate and responsive bottle-feeding behaviours. However, to do so, further studies need to identify the specific bottle-feeding behaviours which may contribute to rapid weight gain amongst infants fed with a bottle. Therefore, cohorts with detailed measures of parental feeding practices in infancy, such as Gemini, would be well placed to investigate specific bottle-feeding practices which may explain, or mediate, the relationship between bottle-feeding and rapid weight development in infancy. Whilst complex interactions between IFMs and feeding practices could be explored in large observational cohorts, studies should also incorporate study designs which are better able to remove confounding from social exposures (e.g. quasi experimental or family designs). Moreover, we postulate that this more inclusive approach to infant feeding guidance – that does not only focus on breastfeeding promotion - might also carry significant benefit for formula feeding families given the lack reputable bottle-feeding guidance available to them at present in UK. Hence, the influence bottle-feeding guidance on infant growth is an important avenue for further research in the field on Infant feeding.

Finally, the DT models presented in the present chapter highlighted the potential for parent-child reciprocity in infant feeding – as twins fed with more bottle or formula feeding were smaller to ~40 weeks of age than their co-twin. I postulate that parents may introduce formula or bottle-feeding in *response* to a twin who is growing more slowly than their co-twin. Whilst many observations studies of IFMs, highlight associations from IFMs to infant growth in the first year of life, such as those highlighted under Aim 3.1 of the current study, few attempt to explore or take account of these bi-directional influences. Specifically, few studies have ensured that the exposure of IFMs truly *precedes* the resulting growth outcomes. Hence, given the observed potential for reciprocity in our DT growth trajectories – I will explicitly explore parent-child reciprocity in IFMs and infant weight development within the next chapter.

3.5.4 Merits and Limitations

The current chapter should be interpreted in line with the various strengths and limitations of these analyses. First, Gemini offers detailed measures of infant feeding modalities, allowing for a more granular measure of IFMs (7-groups) than has often been utilised in previous studies[50,54] This carried numerous benefits, including the ability to distinguish feeding modalities that vary within breastfeeding and identifying a group of infants who were predominantly fed expressed breastmilk from a bottle in the first 3-months of life. Although this group was a small sample (n=39) given the rarity of predominant expressed feeding, it offered a unique opportunity to help understand the mechanism that places formula fed infants at greater risk of RIWG. Moreover, to the authors knowledge, our study is the second study[50] to measure the influence of IFMs on infant growth *trajectories*, as opposed to cross-sectional measures of RIWG. This is important, as presence of RIWG or change in z-score from birth to 12-months only captures change in weight from birth using two weight measurements, as opposed to capturing the pattern of weight gain within the first year of life. Finally, 96.4% of weight measurements at 3 months in Gemini had been taken by a healthcare professional as compared to parent-reported measurements of weight – where small errors in measurement can be common.[91]

Nonetheless, it is crucial to recognise the limitations of this work. Firstly, as aforementioned, a large enough sample of 'exclusive' expressed feeders could not be isolated to power the present LMMS (n=14, not accounting for additional loss of sample due to availability sufficient weight measurements and covariate data) and therefore we included infants fed with predominantly expressed breastmilk and a small amount of formula milk in our 'expressed fed' group. Hence, the small amount of formula milk that may have been provided to these infants may have influenced growth trajectories and increased their similarity to those of infants fed with formula milk. Although this is important to consider as a limitation, it would have not been possible to model growth trajectories for those fed exclusively through expressed breastfeeding. Moreover, as few birth cohorts with repeated measures of infant growth measure both infant feeding methods (breast vs. formula feeding) and modalities in breastfeeding (expressed breastmilk vs. breastfeeding from the breast) we believe these findings are still contribute importantly to the few studies implicating bottle-feeding in more rapid trajectories of weight gain.

Second, twins are on average born at lower birthweight due to foetal restriction.[25] Therefore, twins may have a greater propensity to show RIWG or 'catch up growth' as compared to singletons. This is apparent in the high rates of RIWG (change in weight-for-age z-score > 0.67) at 12-months of age (79.24%) in the sample, as compared to an estimated 40% in the Baby Milk study of singletons fed with formula milk[24]. To counter this limitation, we utilised trajectories of weight for age z-scores, as opposed to change z-score calculations, to detect more nuanced variations in weight gain between infant feeding modalities than possible with cross-sectional measures. Moreover, we excluded pre-term infants (<36 weeks) from the analyses and adjusted for confounding influences, such as time spent in specialist care. Nonetheless, whilst RIWG may be overrepresented in Gemini, the relationship between feeding modalities and infant growth itself is unlikely to vary between singletons and twins, and twins do not differ in their overall long-term health trajectories. Moreover, given the rich phenotyping of growth and infant feeding in Gemini as well as the ability to apply the DT and twin design to this data, it remains useful to explore these research questions in a twin sample. However, future research should seek to confer the current observational findings in singleton samples.

Another important limitation to mention is measurement error in regard to the maternal reports of IFMs. In Gemini, Mothers we're asked at 8-months of age to think back to their feeding methods used across the first 3-months of life – potentially allowing for retrospective recall errors. Moreover, as these IFMs were only reported by mothers – feeding methods more commonly used by other caregivers (e.g. expressed breastfeeding or formula feeding supplementation) by partners or grandparents may be underrepresented.[147] Finally, whilst the current method of IFMs, whilst highly detailed regarding the 7 varying combinations of feeding modality it presents, is less nuanced regarding the ratios of formula to breastfeeding captured within some of these groups. In other words, whilst two twins may have both received 'breastfeeding and formula feeding' the ratio of these two methods might differ significantly, with one twin being fed 'mostly breast and some formula' and the other 'mostly formula and some breast'. These ratios may be important to consider and adjust for in future work seeking to explore the relationship between infant feeding and infant growth.

Numerous limitations with regard to the DT comparisons conducted also need be considered. First, we attempted to retain as much twin-pair discordance as possible under the current DT characterisation (see section 2.3.3). Yet, the sample of discordant twin pairs (n=108 pairs) was relatively small, given the rarity of feeding twins differently in the first 3-months of life, which may have limited the power to detect variations in growth between twins fed discordantly.[35,138] Furthermore, DT comparisons have reduced statistical power in that they rely on fixed-effects models, as compared to mixed-effects, which can result in more imprecise parameter estimates[35]. Hence, the insignificant association between infant feeding and growth trajectories under the DT comparisons might in practice reflect the limited power (n=108) of the comparisons as well as the removal of social confounding. Moreover, discordance between IFMs was represented in a highly sensitive way to retain as much discordance as possible – such that twin pairs who differed in any respect on the 7-group IFM measure were labelled as discordant. However, stronger DT effects may be detected where discordance in IFMs is measured with measures that are able to capture bigger or more marked differences between co-twins – such as volume of formula milk provided or perhaps PFPs. Finally, the important limitation and consideration of DT comparisons is perhaps that parents are unlikely to feed their twins differently unless there is an important difference between the twins or feeding experiences – such as one twin experiencing health complications or weight faltering. Hence, whilst the DT design can remove confounding from shared environmental exposures shared by twins, it cannot remove confounding from these non-shared characteristics that may influence both infant feeding and infant growth.[35] However, in this way, the DT design can therefore be used to shed light on infant characteristics that may lead to variations in IFMs. Moreover, as the DT trajectories of infant weight gain presented in this study showed a trend towards co-twins who were fed with more formula or bottle feeding being smaller in early infancy, I will continue to utilise the DT design to explore parent-child reciprocity in infant feeding in the next chapter.

3.5.5 Concluding Statement

In summary, infants fed formula milk, both exclusively and in combination with breastmilk, showed observationally steeper weight gain trajectories to 12-months of age in Gemini. Moreover, infants fed with expressed breastmilk from a bottle showed steeper weight gain than those fed from the breast. Therefore, bottle-feeding may, in part, promote faster weight gain in the first year of life. Hence, RIWG prevention might also be achieved through interventions promoting appropriate and responsive bottle-feeding behaviours amongst infants receiving formula milk, either exclusively or in combination with breastmilk, as opposed to a sole focus on breastfeeding promotion. Finally, the application of the DT design demonstrated that twins receiving more formula or bottle feeding then their co-twin showed a trend towards being slightly *smaller* in early infancy. Hence, when removing social confounding, breastfeeding did not show marked benefits for infant growth trajectories. However, the confounding influence of non-shared characteristics is critical to consider within twins fed discordantly. As co-twins fed with more formula feeding or bottle-feeding showed a trend towards being smaller in early infancy, the introduction of formula or bottle feeding itself in fact be a *response* to slower weight gain in early infancy. Therefore, the current findings warrant future investigations of potential parent-child reciprocity in infant feeding. Such investigations will help to both establish more causal inference in the relationship between IFMs and RIWG and help to inform interventions and policies seeking to modify feeding behaviours or support healthy growth from the start of life.

Chapter Four. Study 2: Parent-Child Reciprocity in Infant Feeding Modalities and Infant Weight Development Across the First Year of Life

4.1 Introduction

Given the consistent association between formula feeding and higher presence of RIWG across observational studies[11,18] – a view has emerged whereby infant formula and formula feeding is a *cause* of RIWG gain in infancy.[40] Through this mechanism, RIWG may predispose infants to be at a higher risk of developing obesity in later life. These associations have contributed to the adoption of the WHO Growth Standards[11]; a set of optimal growth references derived using a sample of EBF infants, as well as recommendations and policies (e.g. The Baby Friendly Initiative)[139] to exclusively breastfeed to 6-months of age. However, in recent years, researchers have begun to carefully consider how infant feeding might represent a dynamic interaction between caregivers and children. Hence, whilst the majority of infant feeding studies focus on the influence of parents on their children (e.g. how feeding methods influence infant growth)[144] it is possible that child characteristics might, in parallel, influence parental feeding decisions such as IFMs[40].

In recent years, the *reciprocity* hypothesis has emerged, which proposes that the introduction of formula milk may be a *response* to early emerging infant characteristics.[40,70] One important characteristic includes slower infant weight gain or 'weight faltering' in early infancy. Hence, caregivers might introduce formula milk to their infant, either in isolation or combination with breastmilk, in response to slower weight gain or weight faltering in the early weeks of life.[148] This hypothesis therefore challenges the commonly stated cause and effect relationship between infant feeding (the postulated cause) and infant growth (the postulated effect). In line with this hypothesis, it is important to consider how reciprocity may specifically be masked within cross-sectional associations between IFMs and RIWG at 12- or 24-months of age. Specifically, slower weight gain which led to the introduction of formula milk may not be observable in the overall change in weight observed from birth to 6- or- 12-months of life. Emerging studies which have been able to disaggregate early infant weight gain, from later weight gain, indeed observe slower weight gain in early infancy amongst formula fed infants in line with the reciprocity hypothesis.[40,70] For example, a secondary cohort analysis of the Promotion of Breastfeeding Intervention Trial (PROBIT)[39] found that infants given formula milk in their first month of life (n= 1378) were lighter at 1-month of age than infants EBF to either 3 (n=1271) or 6 months (n=251).[41,69] However, to date, only one study has explicitly tested the hypothesis that formula milk introduction is responsive to slower infant growth. Within a subset of infants in the Cambridge Baby Growth Study (CBGS) infants who were EBF from birth, a lower weight gain from 0-2 weeks and 0-6 weeks increased the odds parents introducing formula milk between 2-5 weeks (n=148; OR 0.32; 95% CI 0.12 - 0.77) and 6-11 weeks (n=94; OR 0.18; 95% CI 0.05 - 0.63) respectively[70]. As this is the only study to date which has directly tested this hypothesis – the first aim of the following chapter seeks to further test whether slower infant weight gain during exclusive breastfeeding increases the odds of formula milk introduction in Gemini. In contrast to the CBGS, in Gemini we are able to measure growth in the period *prior* to the introduction of formula milk, as compared more generally across the first 2- or 6- weeks as was done in CBGS. This is possible given the detailed data on timing of formula milk introduction (in days or weeks) and repeated weight measures provided by mothers in Gemini. This helps to further disaggregate growth in the periods of *before* and *after* formula milk introduction to explore the reciprocity hypothesis.

One hypothesised mechanism that may explain why caregivers introduce formula in response to slower weight gain is the concern they have might have for their child's adequate weight gain.[40,142] Hence, it is plausible that parents might introduce formula in response to not only slow weight gain, but also concern for slow weight gain, which has yet to be explored by previous studies. Therefore, we extend the aims of the CBGS to explore whether maternal concern for weight gain in early infancy may also increase the likelihood of formula milk introduction, as a second aim of the study.

Moreover, within a recent secondary analysis of the PROBIT study, infants of mothers who were compliant to the PROBIT intervention (by continued continuing to breastfeed for $\geq$12 months; n=16,086) showed steadily higher weight in the first two months of infancy[40]. Hence, the authors suggest that compliance (continuation of breastfeeding) was a response to their infant's more steady and higher weight patterns in the early weeks of life. However, after 2-months of age, infants of un-compliant mothers (who had introduced formula milk) demonstrated an increase in their rate of weight gain – slightly exceeding the final weight of infants whose parents were compliant (continued to breastfeed). This important finding highlights how despite formula feeding being a response to slower weight gain, it carries the potential to speed up weight gain once introduced. Hence, it is critical to disentangle infant growth trajectories *before* and *after* the introduction of formula milk – as distinct growth patterning might present themselves. The current study is the first, to our knowledge, to disaggregate infant weight gain trajectories before and after the introduction of formula milk.

For the final aim of this chapter, I applied the DT design to explore reciprocity in infancy feeding. Within the previous Chapter Three of this thesis, weight gain trajectories of twin discordant for infant feeding pointed towards important bidirectionality in infant feeding, such that twins fed with more formula or bottle feeding showed slower weight gain trajectories in early infancy. Moreover, another recent DT analyses of Gemini highlighted how twin-pair differences in appetitive traits were associated with differential use of pressuring feeding practices at both 16-months and 5-years of age.[82] Therefore, mirroring these DT approaches within the infancy period can help to further establish infant characteristics that might lead to variations in feeding across a twin pair – a unique quasi-experimental design to highlight reciprocity in infant feeding.

Taken together, very few studies have explicitly explored parent-child reciprocity in the context of IFMs – particularly not applying quasi-experimental approaches such as the twin design. Nonetheless, there is a burgeoning evidence base demonstrates reciprocity between PFPs and child characteristics in later toddlerhood and childhood[73,82,149] (see section 1.2.4 of Chapter One). Therefore, it is plausible that formula feeding may be, in part, a response to infant weight gain or concern for weight development in early infancy.

4.2 Aims

The overall objective of chapter four is to test the reciprocity hypothesis in Gemini, or specifically the extent to which the introduction of formula milk may be a response to slow weight gain in early infancy. To achieve this objective, I utilise bi-directional epidemiological analysis with the twin design to i) measure whether the introduce of formula milk is a response to slower weight gain or maternal concern for slower weight gain during the early weeks of infancy ii) explore whether the introduction of formula milk influences weight gain trajectories after its introduction and iii) explore whether early weight gain or maternal concern for weight gain leads to twin-pair discordance in infant feeding. The specific aims and research questions are outlined below.

Aim 4.1: Explore whether the introduction of formula milk is a response to slower weight gain or maternal concern for weight gain during exclusive breastfeeding

RQ 1; Does slower weight gain during exclusive breastfeeding increase the odds of formula milk introduction?

- ➔ *Hypothesis: Slower weight gain during exclusive breastfeeding will increase the odds of formula milk introduction, compared to continued EBF to ~3-months of age*

RQ 2; Does higher maternal concern for low weight gain from 0-3 months increase the odds of formula milk introduction?

- ➔ *Hypothesis: Higher maternal concern for low weight will increases the odds of formula milk introduction, as compared to continued EBF to ~3-months of age*

Aim 4.2: Explore weight gain trajectories across IFMs before and after the introduction of formula milk

RQ 3; Do exclusively breastfed, and formula fed infants show varying weight gain trajectories in the period *prior to* the introduction of formula milk?

- ➔ *Hypothesis: Infants introduced to formula milk will show slower weight gain trajectories prior to the introduction of formula milk, as compared to infants who continued to be EBF to ~3-months of age*

RQ 4; Do exclusively breastfed and formula fed infants show varying weight gain trajectories *following* the introduction of formula milk?

➔ *Hypothesis: Infants who were introduced to formula milk will show steeper weight gain following the introduction of formula milk, as compared to infants who continue to be EBF to ~3-months of age*

Aim 4.3: Explore if anthropometric characteristics or maternal concern for low weight gain are associated with twin-pair discordance in formula feeding

RQ5; Do anthropometric characteristics or maternal concern for low weight gain vary across twins discordant for formula feeding?

➔ *Hypothesis: Co-twins fed with more formula feed will show lower weight in early infancy and higher reported maternal concern for low weight gain from 0-3 months*

4.3 Methods

4.3.1 Measures

4.3.1.1 Infant Feeding Modality

Information on infant feeding methods was collected when infants were ~8-months (M=8.2 SD=±2.2 months). These measures are described in section 2.2 of Chapter Two. Responses were aggregated to create a binary measure of infant feeding modality: 'Breastfed' (pertaining to exclusive breastfeeding, both from the breast and expression) and 'Formula Fed' (pertaining to both exclusive formula feeding from 2-weeks of life, or formula in combination with breastmilk from the breast or expression from 2-weeks of life). Timing of formula milk introduction was collected by asking mothers: 'How soon after birth did you start bottle-feeding your twins?', with response options in minutes, hours, or days. The question was asked separately for each twin. Partial formula feeding was not separated from exclusive formula feeding for two reasons i) to retain enough power to explore growth trajectories across infants introduced to formula after 2-weeks of age and ii) I was interested in exploring both whether the 'swap' to exclusive formula feeding or supplementation with formula might be a response to slower early growth.

4.3.1.2 Discordance in Formula Feeding

The 7-group infant feeding measure was used to define discordance on formula feeding for the main DT models. Twin pairs where one twin was fed more formula-feeding than their co-twin were classified as discordant on 'formula feeding' (n=62, 4% of term ($\geq$36 week) twin pairs) for the main DT analyses. However, for a sensitivity analysis seeking to increase the power of DT comparisons, we included twin pairs who were discordant for 'expressed breastfeeding or formula feeding' such that one twin was fed more expressed breastfeeding or formula feeding than their co-twin (n=139, 8.86% of term twin pairs). See Section 2.2.3 of Chapter Two for further details as to how these two measures of discordance were generated.

4.3.1.3 Infant Anthropometrics

Collection of anthropometric data from mothers is described in section 2.3.1 in Chapter Two. Weight data were cleaned in line with the procedures outlined by Jaarsveld et al[150].

4.3.1.3.1 Change in Weight for Age Z-Score and Anthropometric Parameters

Weight measurements, alongside the infants' gestational age, postnatal age, and sex, were used to calculate weight-for-age z-scores at each week of postnatal life using the zAnthro growth macro package in Stata[125] (using the UK-WHO Growth Standards). Where multiple measurements were available within a single week, the closest measurement (i.e. at day 7 for 1 week) was used. Change in weight for age z-scores (change z-scores) up to the point of introduction of formula milk for the logistic regression models were calculated by subtracting an infant's birthweight z-score from their z-score at the start of the week in which formula was introduced. For EBF infants, this measure contained change z-scores to 6 weeks of life, chosen as a comparable timepoint as the most common and median week of formula milk introduction amongst formula fed infants. Anthropometric parameters used to predict discordance were weight for age z-score at birth; 2, and 6 weeks; and change in z-score between birth to 6-weeks and 3-months of age.

4.3.1.3.2 Weight Gain Trajectories

Weight for age z-scores were calculated for each week of life to 52 weeks (~1 year) where data was available. An adjusted age variable was then generated, subtracting the week at which formula was introduced from the week of the age measurement. This was necessary for the subsequent LMMs, and allowed for baseline (0) to be centred at the week of formula milk introduction, such that the x-axis represented the week prior to (-6 to 0) and following (0 to 52) the introduction of formula. For breastfed infants, the week of introduction (0) was set at 6 weeks, as both the most common point and median week of formula milk introduction in formula fed infants. Adjusted age measurements of less than -6, were recoded as having been taken at -6 weeks given the small amount of weight data from this period (1.47% of available weight data (n=85 weight data points) and for the LMMs to directly compare the trajectories of breast to formula fed infants prior to and following the introduction of formula. A full description of these Splined LMMs is provided in the Section 2.4.2 of Chapter Two.

4.3.1.3.3 Maternal Concern for Low Weight Gain

Maternal concern for low weight gain from 0-3 months was measured by the question: 'Have you ever been concerned that your baby wasn't gaining enough weight?' with response options being 'yes or 'no' for each twin. Moreover, a follow up question asking mothers to specify the time at which they were concerned with the options; 0-3 months, 4-6 months, 7-9 months, 0-12 months, or older than a year. In this analysis, 0-3 months of age was the period under consideration and the variable represented mothers reporting any concern ('yes') for either of their twins.

4.3.1.3.4 Covariates

For the Logistic regression models and splined LMMS, Infant related covariates included: birthweight z-score, zygosity (dizygotic or monozygotic); sex, gestational age (in weeks) self-reported by mothers at the baseline questionnaire; days spent in specialist care (0 days; 1-4 days; 5-9 days; 10 or more days); timing of introduction to solid food (<4 months; 4 to <5 months; 5 to <6 months; ≥6months) and the postnatal week in which formula was introduced (in weeks). Self-reported family-level covariates included: maternal age at twin delivery (in years); mode of delivery (caesarean or vaginal); maternal BMI (kg/m2), calculated using self-reported weight and height at the time of the baseline questionnaire; maternal ethnicity (white or non-white); maternal marital status at baseline (married; divorced or separated; single); maternal smoking during pregnancy (yes or no), parity (none; one; more than one); presence of gestational diabetes (no; yes); and a composite measure of socioeconomic status (SES) to adjust for numerous indicators of both the family and community level SES.[122] Covariate selection procedures and measurement details are described in Section 2.4.4 of Chapter Two.

For the DT models, covariates included twin pair *differences* in the variables reported in the baseline questionnaire including; sex; birthweight; days spent in specialist care. The continuous difference between co-twins (e.g., birthweight z-score: 0.32) or binary difference (e.g. sex difference: 0=same-sex; 1=different sex) were then added to the adjusted DT models.

4.3.2 Samples

4.3.2.1 Sample for Aim 4.1: Logistic Regression Models

The analytic sample included term ($\geq$36) weeks infants who were either i) EBF (n=353 for the adjusted change z-score model or n=373 for the adjusted maternal concern for low weight gain model, 73.7% and 63.8% of the analytical sample) or ii) formula fed, either exclusively or in combination with breastfeeding, where formula was introduced after two weeks of age (n=130 for the adjusted change z-score model and n=212 for low weight gain model, 26.30% and 36.23%) as described in **Table 4.1**. Infants introduced to formula within the first two weeks of life (83.58% of infants fed with any formula milk from baseline Gemini sample) were excluded, allowing for formula feeding to be a response to infant growth in line with the research questions.

4.3.2.2 Sample for Aim 4.2: Linear Mixed Effects Models (LMMs)

The analytic sample comprised infants who fulfilled the same criteria as the analytic models above and had sufficient weight data for longitudinal growth modelling; including at least two weight z-score measurements before the introduction of formula milk (or 6 weeks of age for EBF infants) and three measurements following formula introduction up to 52 weeks of age for all adjusted LMMs, as informed by previous growth trajectory models investigating IFMs and infant growth.[50,70]

4.3.2.3 Sample for Aim 4.3: Discordant Twin (DT) Models

Twin pairs who showed within pair difference in formula feeding as described in **Table 4.2**, were classified as discordant. As a sensitivity analysis, twin pairs who were discordant for Infant Feeding Modality more generally (i.e. one twin received more expressed breastmilk or formula milk than the other) used to classify discordance, to increase the sample size and statistical power of the fixed-effects models.

4.3.3 Statistical Analyses

4.3.3.1 *Logistic Regression Models*

Logistic regression models were used to test the hypothesis that i) lower change z-score (continuous) from birth to the introduction of formula milk or ii) maternal concern for low weight gain between 0-3 months (binary; yes, no) would predict a higher odd of being introduced to formula milk (binary; yes, no). Models were fitted using maximum likelihood estimation (MLE) and model fit was evaluated using Akaike information criterion (AIC) and Bayesian Information Criterion (BIC). Models were adjusted for the covariates specified above and clustering within families. Statistical significance was set at $p<0.05$ and placed emphasis on effect sizes rather than p-values.

4.3.3.2 Linear Mixed Effects Models (LMMs)

Splined LMMs with MLE were used to model weight z-score trajectories prior to and following the introduction of formula milk. The 'spline' was centred at the point of formula introduction (0), such that estimates were generated for the period 'before' (-6 to 0 weeks) and 'after' the introduction of formula (0 weeks to ~52 weeks). This 'spline' was centred at 6 weeks for EBF infants as the most common and median week of formula introduction for formula fed infants. First, to explore differences in weight for age z-score trajectories between IFM groups *before* the introduction of formula milk, an interaction term between the 'week prior the introduction of formula milk' and 'IFM group' was added to a LMMs model. Then, to explore differences weight for age z-score trajectories between IFM groups *after* the introduction of formula milk, an interaction term between the 'week following the introduction of formula milk' and 'IFM group' was added to a separate LMMs model. β values (with 95% CI's) are reported and statistical significance was set at $p<.05$. Results are reported in full to focus on effect sizes rather than p-values. The same covariates were adjusted for as the Logistic Regression models, with the addition of the postnatal week at which formula milk was introduced. Gestational age (in weeks) was removed as a covariate as this variable was colinear with adjusted age. Akaike information criterion (AIC) and Bayesian information criterion (BIC) were used to determine model fit.

4.3.3.3 Discordant Twin (DT) Models

T-tests and Cohen's D were used to test mean differences in; weight for age z-score at birth; weight z-score at 2 and 6 weeks; change in z-score between birth and 3-months of age, between twin pairs discordant for formula feeding (n=62). For maternal concern for low weight gain from 0-3 months, the number and percentage of infants experiencing this measure are presented alongside binary logistic regressions to test the difference between discordant twins. For the discordant twin models, a secondary analysis was conducted including a broader definition of discordance IFM, including co-twins discordant for expressed breastfeeding and bottle feeding (n=139). This increased the sample of discordant twin pairs and is described in **Table 2.3** (Section 2.2.3). Results are presented alongside the main DT models. Covariates were adjusted for as described in section 4.3.1 above.

4.3.3.4 Sensitivity analyses

Pre-specified sensitivity analyses were conducted. First, repeating the adjusted Logistic Regression models and LMMs without adjustment for covariates (crude models). Second, repeating the adjusted Logistic Regression models and LMMs with exclusion of infants fed with expressed breastmilk from both the EBF and formula or mixed fed groups to explore whether early infant growth influences the introduction of formula milk independently of expressed feeding through a bottle.

4.4 Results

4.4.1 Sample Characteristics

The analysis samples for the Logistic Regression Models (N=483 and N=585) and LMMs (N=450) with their respective characteristics are displayed in **Table 4.1**. A high proportion of the infants, 73.08% and 63.76% (Logistic Regressions) and 72.67% (LMMs), were exclusively breastfed, as expected given the exclusion of infants introduced to formula milk prior to 2-weeks of age. A high proportion of infants experienced RIWG from 0-12-months of age across all of the analysis samples (e.g. 76.45% of Logistic Regression Sample) which was comparable to the baseline samples (69.44%). All three analysis samples showed a similar distribution as compared to the baseline sample of; zygosity, sex, maternal ethnicity, timing of introduction to solid foods and parity. However, these analytic samples showed lower rates of low NS-SEC socioeconomic status (Weight-for-age z-score logistic regression; 9.32%, concern for low weight gain logistic regression; 9.91%, LMMs; 8.67%) as compared to the baseline Gemini Sample (20.88%). Presence of RIWG was high in all samples at both 3-months (e.g. 43.33% in LMMs) and 12-months (e.g. 76.36% in LMMs). Moreover, clinically relevant weight faltering (change z-score <-0.67) was similar across samples at 3-months of age (e.g. 15.41% in LMMs). Finally, maternal concern for low weight was similar across all samples (e.g. 21.72% in LMMs), and concern for overweight was rare across all samples (e.g. 1.81% in LMMs).

Table 4.1. Descriptive Statistics of Baseline Sample of Gemini Infants, Infants Included in Logistic Regression Models and LMMs

		Baseline Sample of Infants (n=4,804)		Individual Twins in Logistic Regression Sample with Change Z-score (N=483)		Individual Twins in Logistic Regression Sample with Concern for Low Weight Gain (N=585)		Individual Twins in Splined LMMs Sample (N=450)	
		N	Mean (SD) or %	*N*	Mean (SD) or %	*N*	Mean (SD) or %	*N*	Mean (SD) or %
Infant Feeding Exposures									
Binary Infant Feeding Method[a]									
	Exclusively Breastfed	654	14.90***	353	73.08***	373	63.76***	327	72.67***
	Mixed Fed or Formula Fed	3,734	85.10	130	26.92	212	36.24	123	27.33
Week at Which Formula Was Introduced for Mixed or Formula Fed Infants									
	1	2,653	74.38	-	-	-	-	-	-
	2	328	9.20	-	-	-	-	-	-
	3	177	4.96	20	15.38	56	26.42	20	15.38
	4	101	2.83	14	10.77	33	15.57	14	10.77
	5	107	3.00	25	19.23	40	18.87	23	19.23
	6	69	1.93	29	22.31	33	15.57	29	22.31
	7	30	0.84	12	9.23	13	6.13	12	9.23
	8	43	1.21	13	10.00	18	8.49	13	10.00
	9	25	0.70	10	7.69	12	5.66	9	7.69
	10	17	0.48	3	2.31	3	1.42	1	2.31
	11	6	0.17	0	0.00	0	0.00	0	0.00
	12	11	0.31	4	3.08	4	1.89	2	3.08
Infant Feeding Modality[b]									
	Exclusively Breastfed	543	12.37***	334	69.15***	352	60.17***	309	69.15***
	Breastfed and Expressed Fed	151	3.44	15	3.11	18	3.08	14	3.11

	Expressed Fed	207	4.72	15	3.11	19	3.25	15	3.11
	Breastfed and Formula Fed	1,201	27.37	103	21.33	174	29.74	96	21.33
	Breast, Formula Fed and Expressed Fed	259	5.90	11	2.28	13	2.22	11	2.28
	Formula Fed and Expressed Fed	393	8.96	3	0.62	4	0.68	5	1.11
	Formula Fed	1,634	37.24	-	-	-	-	-	-
Infant & Familial Characteristics									
Zygosity									
	Dizygotic	3,232	68.33	348	72.05	425	72.65	332	72.33
	Monozygotic	3,232	31.67	135	27.95	160	27.35	118	27.95
Sex									
	Boy	2,386	49.67	213	44.10	269	45.98	197	43.78
	Girl	2,418	50.33	270	55.90	316	54.02	253	56.22
Maternal Ethnicity									
	White	4,462	92.96	461	95.45	563	96.24	430	95.56
	Other	338	7.04	22	4.55	22	3.76	20	4.44
Term									
	Under 35 Weeks	1,382	28.89	-	-	-	-	-	-
	Over or 35 Weeks	3,402	71.11	483	100	585	100	450	100.00
Gestational Age (in weeks)		4,784	36.20 (2.48)	483	37.71 (1.18)	585	37.62 (1.14)	450	37.68 (1.18)
Birthweight z-score[c]		4,639	-.87 (0.96)	483	-1.15 (0.85)	585	-1.17 (0.84)	450	-1.17 (0.85)
Rapid Growth at 3-months[d] (z-score >0.67)									
	Weight Faltering (<-0.67)	652	15.41	62	13.45	70	12.82	59	13.11
	No Rapid Weight Gain	1,682	39.74	200	43.38	228	41.76	196	43.56
	Rapid Weight Gain	1,898	44.85	199	43.17	248	45.42	195	43.33
Rapid Growth at 12-months[d] (z-score >0.67)									
	Weight Faltering (<-0.67)	156	6.98	4	1.54	4	1.25	4	1.55
	No Rapid Weight Gain	527	23.58	57	22.01	62	19.44	57	22.09
	Rapid Weight Gain	1,552	69.44	198	76.45	253	79.31	197	76.36
Mode of Delivery									

	Caesarean	3,118	65.20	254	52.59	329	56.24	239	53.11
	Vaginal	1,664	34.80	229	47.41	256	43.76	211	46.89
Days in Specialist Care									
	0 days	3,622	76.32	397	82.19	481	82.22	367	81.56
	1 – 4 days	593	12.49	64	13.25	74	12.65	61	13.56
	5 – 9 days	212	4.47	14	2.90	23	3.93	14	3.05
	10 + days	319	6.72	8	1.66	7	1.20	8	1.74
Introduction of Solid Foods Timing									
	≥6 months	1,105	24.37	138	28.57	155	26.50	126	28.10
	5 to< 6 months	1,444	31.85	179	37.06	215	36.75	167	36.82
	4 to <5 months	1,385	30.55	130	26.92	164	28.03	122	27.45
	<4 months	600	13.23	36	7.45	51	8.72	35	7.63
Parity									
	None	2,398	51.42	203	42.03	257	43.93	200	44.01
	One or More	2,266	48.58	280	57.97	328	56.07	250	55.99
National Statistics Socioeconomic Class (NS-SEC)[e]									
	High	2,662	61.84*	368	76.19*	448	76.59*	346	76.89*
	Middle	744	17.28	70	14.49	79	13.50	65	14.44
	Lower	899	20.88	45	9.32	58	9.91	39	8.67
Index of Multiple Deprivation[f]									
	Least Deprived	950	19.98*	95	19.67	113	19.32	118	26.22*
	Not Deprived	952	20.23	119	24.64	151	25.81	130	28.89
	Mid	950	19.46	119	24.64	151	23.93	105	23.33
	Deprived	952	20.03	88	18.22	109	18.63	71	15.78
	Most Deprived	950	19.98	126	12.84	72	12.31	26	5.78
Maternal Concern Surrounding Infant Weight									
Concern about Low Weight Gain between 0-3 Months[g]									
	Yes	163	24.22	101	21.26	121	20.68	96	21.72
	No	510	75.78	374	78.74	464	79.32	346	78.28
Concern about High Weight Gain between 0-3 Months[g]									
	Yes	99	2.12	9	1.90	9	1.56	8	1.81
	No	4,579	97.88	465	98.10	569	98.44	433	98.19

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.

[a]Exclusively Breastfed: Fed exclusively breast milk from the breast; Breastfed and Expressed Fed : Fed with a combination of breast milk from the breast and expressed milk in a bottle; Expressed Fed: Fed with expressed milk in a bottle; Breastfed and Formula fed; Fed with a combination of breast milk from the breast and formula milk; Breast, Formula and Expressed: Fed with a combination of breast milk from the breast, expressed milk in a bottle, and formula milk; Formula and Expressed Fed: Fed with a combination of expressed milk from a bottle and formula milk; Formula Fed: Fed with predominantly formula milk

[b]Exclusively Breastfed: Fed exclusively breast milk from the breast; Breastfed and Expressed Fed: Fed with a combination of breast milk from the breast and expressed milk in a bottle; Expressed Fed: Fed with expressed milk in a bottle; Breastfed and Formula fed; Fed with a combination of breast milk from the breast and formula milk; Breast, Formula and Expressed: Fed with a combination of breast milk from the breast, expressed milk in a bottle, and formula milk; Formula and Expressed Fed: Fed with a combination of expressed milk from a bottle and formula milk; Formula Fed: Fed with formula milk

[c] Weight for Age Z-Scores: Calculated using UK-WHO growth refence data adjusting for age, sex, and gestational age.

[d] Change in weight for age z-scores; Z-score at birth subtracted from z-score at 12- or 3- months of age

[e] Household NS-SEC based on the Higher Ranking Position of the two full NS-SECs

[f]5 quintiles of deprivation, 1 = 'score ≤8.49 (least deprived quintile)', 2 = '8.5–13.79', 3 = '13.8–21.35', 4 = '21.36–34.17', 5 = '≥ 34.18 (most deprived quintile)'

[g] Have you ever been concerned that your baby wasn't gaining enough weight/was gaining to much weight, followed by a question asking mothers to specify the time at which they were concerned (0-3 months of age)

% for analytic sample do not include missing data to represent distribution of variables across infants included in the sample

The samples for the twin pairs discordant on formula feeding (n=62) and infant feeding modalities (n=139) alongside the baseline sample of Gemini twin pairs are reported **in Table 4.2**. A higher proportion of the twins in the discordant feeding twin samples were discordant for sex, 51.08% (IFM discordance) and 56.45% (Formula Feeding Discordance) as compared to the Baseline sample of Gemini Twin pairs (33.97%). Similarly, the discordant samples had a higher proportion of dizygotic pairs, 83.82% (IFM discordance) and 88.33% (Formula Feeding Discordance) as compared to the Baseline Gemini sample (68.33%). Moreover, the sample of twins discordant for formula feeding had slightly higher proportions of caesarean births (80.65%) as compared to the baseline sample (65.20%). Finally, the discordant sampled showed similar distributions of NS-SEC and Index of Multiple Deprivation as compared to the baseline Gemini sample.

Table 4.2. Descriptive Statistics of Baseline Sample of Gemini Twin Pairs Compared to Discordant Twin Samples

		Baseline Sample of Gemini Twin Pairs (n=2404)		Twin Pairs Discordant in Infant Feeding Modalities[a] (n=139)		Twin Pairs Discordant in Formula Feeding[b] (n=62)	
		N	Mean (SD) or %	*N*	Mean (SD) or %	*N*	Mean (SD) or %
Infant & Familial Characteristics							
Zygosity							
	Dizygotic	1,616	68.33***	114	83.82***	53	88.33***
	Monozygotic	749	31.67	22	16.18	7	11.67
Sex Discordance							
	Male, Male	785	32.68**	24	17.27**	15	24.19**
	Female Female	801	33.35	44	31.65	12	19.35
	Male, Female	816	33.97	71	51.08	35	56.45
Ethnicity							
	White	2,231	92.96	133	95.68	60	96.77
	Other	169	7.04	6	4.32	2	3.23
Gestational Age (in weeks)		2,392	36.20 (2.48)	139	37.37 (0.95)	62	37.45 (0.92)
Mode of Delivery							
	Caesarean	1,559	65.20*	97	69.78	50	80.65*
	Vaginal	832	34.80	42	30.22	12	19.35
Parity							
	None	1,199	51.42	83	59.71	36	58.06

	One or More	1,133	48.58	52	37.41	24	38.71
National Statistics Socioeconomic Class (NS-SEC)[c]							
	High	1,515	63.28	93	66.91	41	66.13
	Middle	470	17.00	21	15.11	9	14.52
	Lower	472	19.72	24	17.27	11	17.74
Index of Multiple Deprivation[d]							
	Least Deprived	475	19.98	22	16.55	10	16.13
	Not Deprived	476	20.03	26	23.74	10	16.13
	Mid	475	19.98	32	23.02	17	27.42
	Deprived	1,515	20.03	33	18.71	13	20.97
	Most Deprived	475	19.98	23	15.83	11	17.74

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.

[a] Twin Discordant in Infant Feeding Modality such that one twin was fed with formula milk or expressed breastmilk and one twin was fed was given less formula or expressed breastmilk than their co-twin
[b] Twin Discordant in Formula Feeding such that one twin was fed with formula milk and one twin was fed was given less formula than their co-twin
[c]Household NS-SEC based on the Higher Ranking Position of the two full NS-SECs
[c]5 quintiles of deprivation, 1 = 'score ≤8.49 (least deprived quintile)', 2 = '8.5–13.79', 3 = '13.8–21.35', 4 = '21.36–34.17', 5 = '≥ 34.18 (most deprived quintile)'
% for analytic sample do not include missing data to represent distribution of variables across infants included in the sample

4.4.2 Aim 4.1: Explore whether the introduction of formula milk is a response to slower weight gain or concern for weight gain in early infancy

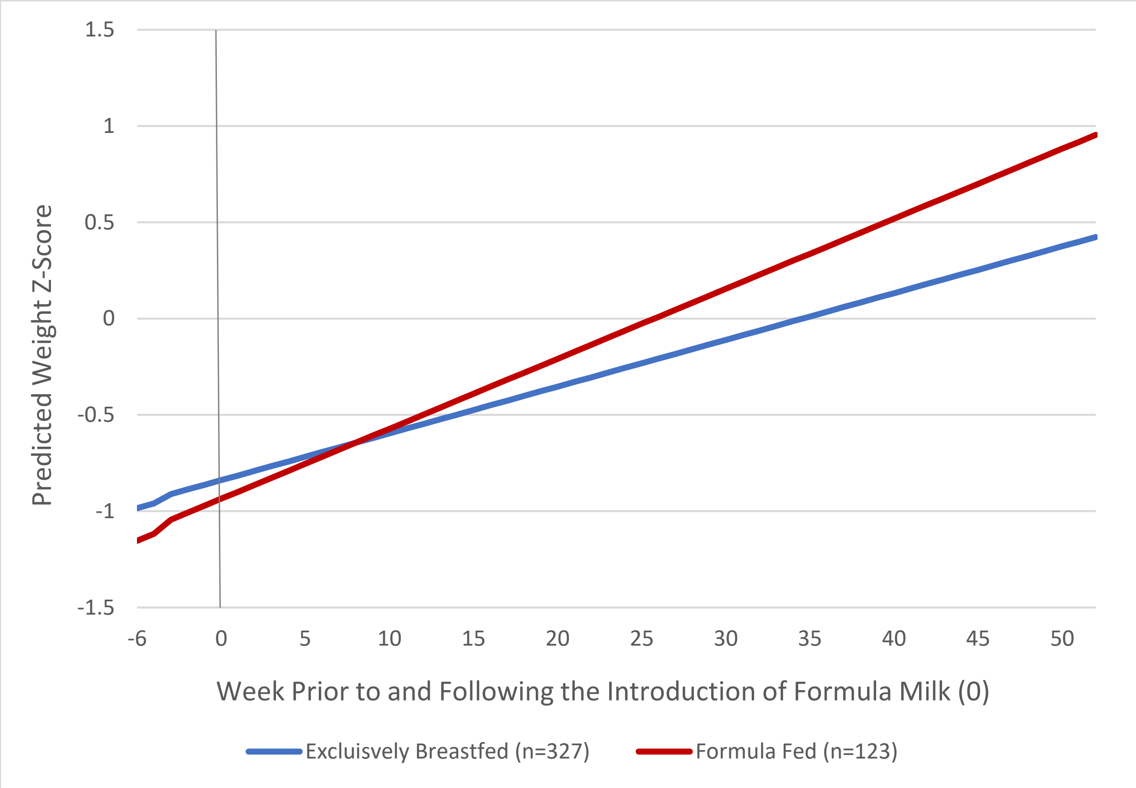
In adjusted Logistic Regression models, higher change in weight z-score from birth to the point of introduction of formula milk, or 6-weeks for EBF infants, reduced the odds of subsequent introduction to formula milk (n=483; OR=0.37; 95% CI=0.24 – 0.57, p<0.001) as compared to continuing to be EBF to ~3-months of age. For each 1 SD higher change in weight z-score prior to the introduction of formula milk, the odds of being introduced to formula milk reduced by 63%. The adjusted model was a better fit to the data (AIC = 517.34 BIC = 621.63) than the unadjusted model (AIC =775.09, BIC = 788.93). A full table of estimates is reported in Appendix IV.

In a separate model, maternal concern for low weight gain from 0-3 months was marginally associated with increased odds of being introduced to formula milk (n= 585; OR=1.75; 95% CI=0.97 - 3.14, p=0.06) as compared to continuing to be EBF to ~3-months of age. The odds of being introduced to formula milk was 75% higher among those mothers who reported concern for low weight gain, as compared to those who did not. The adjusted model was a better fit to the data (AIC = 706.05 BIC = 815.34) than the unadjusted model (AIC =1045.11, BIC = 1059.57). A full table of estimates is reported in Appendix IV. Finally, findings were consistent in sensitivity models without adjustment for covariates (Appendix V) and excluding infants fed through expression (Appendix VI).

4.4.3 Aim 4.2: Explore weight gain trajectories across IFMs prior to and following the introduction of formula milk

During the period of exclusive breastfeeding, or prior to the introduction of formula milk, infants introduced to formula milk showed lower z-score at baseline (β = -0.50 weight-for-age z-score at baseline; 95% CI:- 0.91 - -0.20, p=0.001, **Figure 4.1**, **Table 4.3**) and showed slower rate of increase in z-score per week (β = -0.07 z-score per week; 95% CI:- 0.13 - -0.02, p=0.016, Figure 4.1, Table 4.3) as compared to infants who continued to the exclusively breastfed. Conversely, in the period after the introduction of formula milk (0) up to 52 weeks, infants introduced to formula milk showed steeper z-score trajectories (β = +0.01 z-score per week; 95% CI: 0.00-0.02, p=0.01, Figure 4.1, Table 4.3) as compared to infants exclusively breastfed to ~3-months of age. Hence, infants introduced to formula milk 'caught up' to EBF infants 8-weeks following the introduction of formula milk and thereafter surpassed their EBF counterparts. The linear model was a better fit to the data (AIC = 1584.771. BIC = 1734.333) than a model including a quadratic time term (AIC =1726.998, BIC = 1876.559) and findings were consistent in sensitivity models without adjustment for covariates (Appendix VII) and excluding infants fed through expression (Appendix VIII)

Figure 4.1. Weight for Age Z-Score Trajectories Prior to and Following Introduction of Formula Milk Between Exclusively Breastfed and Formula Fed Infants in Gemini (N=450)



Adjusted for; birthweight z-score, zygosity (dizygotic or monozygotic); sex, self-reported by mothers at the baseline questionnaire; days spent in specialist care (0 days; 1-4 days; 5-9 days; 10 or more days); timing of introduction to solid food (<4 months; 4 to <5 months; 5 to <6 months; >6months); the postnatal age in weeks in which formula was introduced,, maternal age at twin delivery (in years); mode of delivery (caesarean or vaginal); maternal BMI (kg/m2), calculated using self-reported weight and height at the time of the baseline questionnaire; maternal ethnicity (white or non-white); maternal marital status at baseline (married; divorced or separated; single); maternal smoking during pregnancy (yes or no), parity (none; one; more than one); presence of gestational diabetes (no; yes); a composite measure of socioeconomic status (SES) to adjust for numerous indicators of both the family and community level SES, *clustering within families + timing of introduction to formula (set at 6-weeks for BF infants).*

Exclusively Breastfed: Fed exclusively breast milk from the breast; or Fed with a combination of breast milk from the breast and expressed milk in a bottle or Fed with expressed milk in a bottle Formula Fed; Fed with a combination of breast milk from the breast, expressed milk in a bottle, and formula milk; or predominantly formula milk introduced after 2-weeks of age

Predicted Z-Score; Weight-for Age Z-Scores: Calculated using UK-WHO growth refence data adjusting for age, sex, and gestational age. Weeks; Weeks prior to (-6 to 0) the introduction of formula milk with 0 being the week in which formula milk was introduced. 0 set to 6 weeks for Exclusively Breastfed Infants.

Table 4.3. Adjusted Weight for Age Z-Score Trajectories Prior to and Following the Introduction of Formula Milk, Between Exclusively Breastfed and Formula Fed Infants (N=450)

	Adjusted Z-score Trajectories Prior to the Introduction to Formula (N=450)				Adjusted Z-score Trajectories After the Introduction to Formula (N=450)			
	N	β	95% CI	P value	N	β	95% CI	P value
IFM Term; Weight for Age Z-Score at Baseline								
Exclusively Breastfed[b] (ref)	327	-	-		327	-	-	
Formula Fed	123	-0.50	-0.81, -0.20	0.001***	123	-0.14	-0.41, 0.11	0.262
IFMxTime Term; Rate of Change in Weight for Age Z-score Per Week								
Exclusively Breastfed[b] (ref)	327	-	-		327	-	-	
Formula Fed	123	-0.07928	-0.13355, -0.01500	0.01**	123	0.01338	0.00405, 0.02271	0.01**
Cons		0.95	.38, 1.52			-0.25	-1.67, 1.39	

*** p < 0.001; ** p < 0.01; * p < 0.05.

IFM Term; Weight for Age Z-Score at Baseline; Describes the difference in weight z-scores (Dependant variable) between exclusively breastfed (ref) and Formula fed infants (Independent Variable) at birth or the week of formula milk introduction
IFMxTime Term; Rate of Change in Weight for Age Z-score Per Week; Describes the difference in increase or decrease in weight z-scores per week (Dependant variable) between exclusively breastfed (ref) and formula fed infants (Independent variable)

Adjusted for, birthweight, zygosity, sex, days spent in specialist care, maternal age at delivery, mode of delivery, maternal BMI, maternal ethnicity, maternal marital status, maternal smoking during pregnancy, gestational diabetes, composite socioeconomic position, introduction of solid foods, and clustering within families + timing of introduction to formula (set at 6-weeks for BF infants)
Baseline for Prior to the Introduction to Formula was 6 weeks before to the introduction of formula milk (or birth for EBF infants), Baseline for Following the Introduction to Formula was week of formula milk introduction (or 6-weeks of age for EBF infants)
IFM Term; Exclusively Breastfed: Fed exclusively breast milk from the breast; or Fed with a combination of breast milk from the breast and expressed milk in a bottle or Fed with expressed milk in a bottle; Formula Fed; Fed with a combination of breast milk from the breast, expressed milk in a bottle, and formula milk; or predominantly formula milk introduced after 2-weeks of age

4.4.4 Aim 4.3: Utilize the discordant twin design to explore whether anthropometric characteristics or maternal concern for low weight gain led to discordance in formula feeding

When exploring variations in anthropometrics across discordant twins, there was no significant difference in birthweight z scores, weight-for-age z-scores at 2- or 6-weeks or change in z-score from 0-6 weeks or 0-3 months between twin pairs discordant for formula feeding. Whilst not statistically significant, amongst twin pairs discordant for formula feeding (n=62) a higher proportion of mothers reported concern for low weight gain for the twin who received more formula milk than their co-twin (31.5% vs. 20.0%, OR=1.83, p= 0.17, **Table 4.4, Figure 4.2**).

Table 4.4. Differences in Anthropometrics and Maternal Concern for Weight Development Between Twin Pairs Discordant for Formula Feeding (N=62 pairs)

	Twin Fed with Less Formula (n=62)		**Twin Fed with More Formula (n=62)**					
	N (Twins)	Mean (SD) Or n (%)	N (Twins)	Mean (SD) or n (%)	Mean difference	T or Odds Ratio	Cohens D	P value
Z-score at birth[a]	58	-1.49 (0.78)	58	-1.47 (0.91)	-.02	-0.13	-.02	0.89
Z-score at 2 weeks[a]	30	-1.26 (0.67)	30	-1.55 (0.89)	.28	1.41	0.44	0.09
Z-score at 6 weeks[a]	54	-0.63 (1.11)	54	-0.65 (1.04)	.02	0.08	0.02	0.92
Change in Weight Z-Score 0-6 Weeks	54	0.77 (0.85)	54	0.75 (0.82)	0.02	0.12	0.13	0.90
Change in Weight Z-Score 0-3 Months	58	0.75 (1.05)	58	0.60 (1.08)	.14	0.73	0.02	0.47
Maternal Concern for Low Weight Gain 0-3 Months[b] (Yes)	55	11 (20.00%)	55	17 (31.48%)	6	1.83	-0.26	0.17

Formula Feeding Discordance; one twin was fed specifically with more formula feeding than their co-twin (see Chapter Two, Section 2.2.3)
[a]Weight for Age Z-Scores: Calculated using UK-WHO growth refence data adjusting for age, sex, and gestational age.
[b]Concern for Low Weight Gain; This was measured by the question: 'Have you ever been concerned that your baby wasn't gaining enough weight?' with a follow up question asking mothers to specify the time at which they were concerned (response options; ; 0-3 months, 4-6 months, 7-9 months, 0-12 months, or older than a year). N (%) displayed represents mothers who responded 'yes'.
Continuous factors, t-test and Cohens D, Categorical factors, binary logistic regression adjusted for; twin pair differences in sex; birthweight; days spent in specialist care
*** p < 0.001; ** p < 0.01; * p < 0.05.

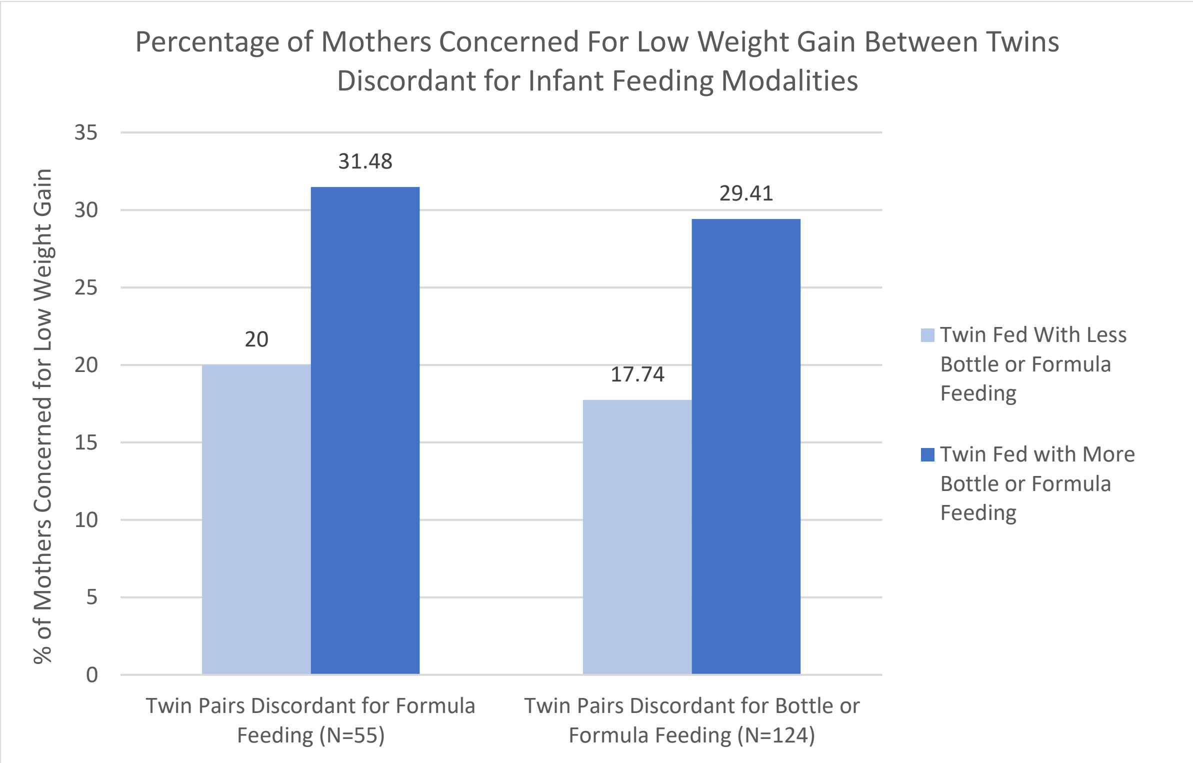
In a second analysis exploring variations in anthropometrics across twins discordant for Infant Feeding Modality, such that one twin was fed with either more formula milk or bottle feeding (expressed breastmilk), we saw a significantly higher proportion of maternal concern for low weight gain for the twin who received more formula or bottle feeding than their co-twin (29.41% vs. 17.74%, OR=1.93, p= 0.03, **Table 4.5**, Figure 4.2). The adjusted model was a better fit to the data (AIC = 260.57 BIC = 277.74) than the unadjusted model (AIC =264.11, BIC = 279.09). However, there was no significant difference in birthweight z scores, weight-for-age z-scores at 2- or 6-weeks or change in z-score from 0-6 weeks or 0-3 months between twin pairs discordant for formula feeding.

Table 4.5. Differences in Anthropometrics and Concern for Weight Development Between Twin Pairs Discordant for Infant Feeding Modality (N=139 pairs)

	Twin Fed with Less Formula or Expressed Feeding (N=139)		**Twin Fed with More Formula or Expressed Feeding (N=139)**					
	N (Twins)	Mean (SD) or n (%)	N	Mean (SD) or n (%)	Mean difference	T or Odds Ratio	Cohens D	P value
Z-score at birth[a]	126	-1.28 (0.84)	126	-1.38 (0.98)	0.10	0.91	0.05	0.36
Z-score at 2 weeks[a]	37	-1.21 (0.72)	37	-1.46 (0.12)	0.24	1.17	0.27	0.24
Z-score at 6 weeks[a]	66	-0.49 (1.15)	66	-0.62 (1.07)	0.12	0.63	0.11	0.52
Change in Weight Z-Score 0-6 Weeks	66	0.84 (0.08)	66	0.73 (0.07)	0.11	0.96	-0.10	0.33
Change in Weight Z-Score 0-3 Months	69	0.70 (1.09)	69	0.53 (1.10)	0.17	0.93	0.16	0.34
Maternal Concern for Low Weight Gain 0-3 Months[b]	124	22 (17.74%)	124	35 (29.41%)	12	1.93**	-.24	0.03*

Twin Pairs Discordant in Infant Feeding Modality; one twin was fed with higher risk IFM (more formula milk or expressed breastmilk) and one twin was fed was lower risk IFM (given less formula or expressed breastmilk than their co-twin)
[a]Weight for Age Z-Scores: Calculated using UK-WHO growth refence data adjusting for age, sex, and gestational age.
[b]Concern for Low Weight Gain; This was measured by the question: 'Have you ever been concerned that your baby wasn't gaining enough weight?' with a follow up question asking mothers to specify the time at which they were concerned (response options; ; 0-3 months, 4-6 months, 7-9 months, 0-12 months, or older than a year)
Continuous factors, t-test and Cohens D, Categorical factors, binary logistic regression adjusted for; twin pair differences in sex; birthweight; days spent in specialist care
*** p < 0.001; ** p < 0.01; * p < 0.05.

Figure 4.2. Percentage of Mothers Reporting Concern for Low Weight Gain (0-3 Months) Between Twins Discordant for Infant Feeding Modalities



Twin Discordant in Infant Feeding Modalities; Formula Feeding Discordance; one twin was fed primarily formula milk and one twin was fed was given less formula (mixed with breastfeeding or expressed feeding, or just breastfed) and Infant Feeding Modality Discordance; one twin was fed with more bottle feeding (expressed breastfeeding) or formula feeding than their co-twin
Concern for Low Weight Gain; This was measured by the question: 'Have you ever been concerned that your baby wasn't gaining enough weight?' with a follow up question asking mothers to specify the time at which they were concerned (0-3 months of age was the period of time included for the analyses).

4.5 Discussion

4.5.1 Summary of Findings

The present chapter triangulated advanced epidemiological analyses with the DT design to explore reciprocity between infant feeding modalities and weight development across the first year of life. Findings largely supported the presence of reciprocity. Hence, the introduction of formula milk may, in part, be a behavioural response towards slower infant weight gain in early infancy and higher concern for low weight gain in early infancy. However, rate of infant weight gain increased amongst the infants subsequently introduced to formula milk, suggesting that formula milk is an effective strategy to promote weight gain following its introduction.

First, this study sought to explore whether slower infant weight gain or maternal concern for low weight gain during exclusive breastfeeding increased the likelihood of parents introducing formula milk (alone or in combination with breastmilk). Both slower infant weight gain during exclusive breastfeeding, as well as maternal concern for low weight gain between birth and 3-months, predicted higher odds of formula milk introduction. However, the findings relevant to concern for low weight gain were only marginally significant.

The second aim of the study was to utilise the repeated measures of weight available in Gemini to disentangle growth trajectories *prior to* and *following* the introduction of formula milk. Conferring the results from aim one, we observed slower growth during the period of exclusive breastfeeding (capturing lower birthweight and slower weight gain trajectories) amongst infants who would be subsequently introduced to formula milk when compared to those who would continue to be EBF to ~3 months of age. Together, these findings confer that the introduction of formula milk may partly be a response to slower weight gain in early infancy. Nonetheless, longitudinal weight trajectory modelling indicated that in the period after formula introduction (or 6-weeks of age for EBF infants) infants fed with formula milk showed significantly steeper rates of weight gain compared to those who continued to be EBF. Specifically, by 8-weeks following its introduction, infants introduced to formula 'caught up' to and began to surpass their EBF counterparts. This is a novel finding, as no studies to date have explored growth patterns amongst formula fed infants in the specific periods surrounding its introduction. As infants appear to gain weight more rapidly in *response* to being supplemented with formula milk, this suggests formula feeding is an effective strategy in increasing weight gain despite earlier reciprocity towards slower growth. Hence, findings from this chapter indicate that infant growth can influence feeding practices and feeding practices can thereafter influence growth – in a dynamic reciprocal process.

In applying DT methods to further explore reciprocity, I did not observe a significant difference in weight at birth, 2 weeks or 6 weeks of age, nor in RIWG to 6-weeks or 3-months of age between co-twins discordant for formula feeding. However, looking to the estimates and effect sizes I observed a trend whereby the twin fed with more formula milk was both smaller and gained less weight to 3-months than their co-twin – consistent with the reciprocity hypothesis. It can therefore be speculated that the reduced statistical power of smaller group DT comparisons, using fixed effects, may have inhibited these inferential comparisons of anthropometric outcomes. Moreover, there was no significant difference in maternal concern for low weight gain between infants fed with discordant for formula feeding. However, once again, we did observe a trend towards higher maternal reported concern for low weight gain in the co-twin who received more formula feeding (31.5% of mothers reported concern) as compared to less formula feeding (20% of mothers reported concern). Moreover, when we expanded the DT sample using the broader measure of IFM discordance (n=139), such that twin pairs where one twin was fed with more expressed breastfeeding through a bottle or formula feeding were included to increase statistical power - the co-twin fed with more bottle or formula feeding showed significantly higher proportions maternal reported concern for low weight gain. Of note, the effect sizes of these broader DT comparisons were similar to those of the formula feeding specific comparisons (Formula Feeding Discordance; d=-0.26, Broader IFM Discordance; d=-0.24). Therefore, I postulate that the former comparisons may have been hindered by the small sample size, and parents may indeed adapt their milk-feeding practices according to their level of concern about their infant's weight gain. Taken together, the provision of formula feeding may be a response to slower weight gain as well as concern for slower growth in early infancy – supporting the presence of reciprocity between IFMs and infant growth.

4.5.2 Comparison of Findings to Previous Literature

4.5.2.1 Aim 4.1: Explore whether the introduction of formula milk is a response to slower weight gain or concern for weight gain in early infancy

In line with the hypotheses - slower infant weight gain and maternal concern for low weight gain predicted higher likelihood of formula milk introduction. Although the findings regarding maternal concern for low weight gain were marginally significant. Overall, these findings confer those of the UK CBGS (n=148), a smaller singleton sample, where slower infant weight gain during EBF predicted higher odds of subsequent formula milk introduction.[70] However, our findings increase the specificity of the growth measurements used by the CBGS. As Gemini collected detailed information on the timing of formula milk introduction, we were able to measure whether rate of weight gain in the period prior to formula milk introduction (specific to each individual formula fed infant) predicted formula introduction. Whereas, in the CBGS - weight gain in two *general* periods – from birth to 2-weeks or 6-weeks – was used to predict the odds of formula introduction. Overall, the higher individuality of this measure helps to disentangle growth trajectories in these two important and distinct periods. Moreover, these results also align with a recent analysis of the PROBIT study – where infants who were breastfed (any breastfeeding) for $\geq$12 months showed higher weight in the first two months, as compared to infants who were introduced to formula.[40] Specifically, infants introduced to formula milk, and therefore 'uncompliant' with the PROBIT intervention, showed a drop in weight z-score between birth to 2-months of age, suggesting that the supplementation of formula may have been a response to slow early weight gain or weight faltering. However, the present steady tested this hypothesis more directly using the first Logistic Regression Models.

The authors of these previous studies speculate that this behavioural response towards slow weight gain, may also be in part due to the parental concerns surrounding slower weight gain or weight faltering.[40,41] Moreover, it has been speculated that concern felt by caregivers could be somewhat heightened by the current UK-WHO growth standards.[11] These growth standards represent the growth of EBF infants who meet a strict set of socio-economic and health related criteria – and therefore show higher mean weight in the first few months when compared to the CDC standards of infants also given formula milk.[41,112] Therefore, these 'inflated' growth reference may be heightening parental concerns surrounding adequate weight gain and may contribute to the decision to supplement with formula milk. In testing this hypothesis, this study demonstrates that that self-reported maternal concern for low weight gain in early infancy increased the likelihood of mothers introducing formula milk. This suggests that parental perceptions and concerns surrounding infant growth may play an important role in parent-child reciprocity in infant feeding. Nonetheless, alternative mechanisms which may contribute to the introduction formula milk in the face of slower growth also need be considered. For example, qualitative insights from parents highlight a pressure or encouragement from health professionals to supplement with formula milk which should be considered by future studies.[151] This encouragement, may also be influenced by the 'inflated' UK-WHO growth references. Nonetheless, the present findings highlight important but scarcely considered parent-child reciprocity in infant feeding. These findings also mirror demonstrated parent-child reciprocity in later childhood, in relation to PFPs and child weight development – see section 1.2.4 of Chapter One for a review of this literature.[71,72,81,82,113] Hence, we postulate that parents may adapt both *what* (e.g. IFMs) and *how* (e.g. PFPs) an infant is fed based on their infant's weight development. As these dynamic processes begin as early the first few weeks of life, they warrant future attention from the field of infant and child feeding research.

5.5.2.2 Aim 4.2: Explore weight gain trajectories across IFMs prior to and following the introduction of formula milk

For the second aim of this chapter, I used longitudinal trajectory modelling to disentangle growth trajectories *prior to* and *following* the introduction of formula milk. Mirroring the findings under Aim 4.1, I observed that infants who would go on to be introduced to formula milk gained weight slower than infants who were EBF to 3-months within the period prior to formula milk provision. Once again, these results suggest that caregiver introduce formula milk partly in response to slower weight gain in the early weeks of life. However, in the period after the formula milk introduction, infants fed with formula milk, either in isolation or combination with breastmilk, showed significantly steeper rates of weight gain to 12-months of age, as compared to those who continued to be EBF. Hence, these infants 'caught up' to their EBF counterparts by 8-weeks after formula milk introduction and thereafter surpass them. Therefore, our findings also support the hypothesis that formula milk may promote rapid weight gain independent of a previous period of EBF. Whilst few studies have been able to disaggregate growth in this way – our growth trajectories mirror those of a PROBIT analyses.[40] Hence, infants whose mothers were 'uncompliant' with PROBIT (or did not continue to breastfeed for ≥12 months) showed lower weight in the first two months of infancy. However, thereafter, they showed an increase in their speed of weight gain to slightly exceeded the final weight of infants who were breastfeed (for ≥12-months) at 12-months of age. Together, my findings confer the distinct growth patterns measured in these distinct periods of infancy. However, it is noteworthy that in Gemini, infants introduced to formula milk significantly exceeded the weights of those who continued to be EBF (to 3-months) at 12-months of life, whereas in the PROBT results this difference was less marked. This suggests that rapid weight gain which occurs following formula milk introduction might not only reflect 'catch up' growth (to compensate for earlier weight faltering) but contribute to weight gain beyond EBF counterparts in the longer term. Moreover, we postulate that infants in Gemini exceeded the weights of their EBF counterparts sooner and more significantly due to the higher use of formula feeding in a twin sample generally, as well as the varied measures of 'breastfeeding'. As Gemini's breastfed comparison group represented EBF to 3-months, as opposed to 'any' breast feeding for ≥12 months in the PROBIT study, I postulate that we were able to observe greater differences in growth trajectories between IFM groups due to less 'spill over' of formula feeding.

In summary, the present study confers the presence of parent-child reciprocity in infant feeding. IFMs are influenced by early infant weight gain, such that formula milk supplementation is, in part, a response to slower growth and maternal concern for low weight gai. However, rate of weight gain following supplementation is also modified by IFMs – such that formula milk encourages more rapid weight gain after its introduction.

5.5.2.3 Aim 4.3: Utilize the Discordant Twin design to explore whether anthropometric characteristics or maternal concern for low weight gain led to discordance in formula feeding

The final aim of this chapter was to utilise the DT design to investigate whether differences in anthropometric characteristics or concern for low weight gain across a twin pair was associated with discordant infant feeding. Results showed that mothers were more likely to report concern for low weight gain for the twin receiving more formula milk than their co-twin. However, this association was not statistically significant, likely due to the small sample of twins who were discordant for formula feeding (n=62 pairs). Hence, when a larger sample of infants fed discordantly on IFMs (such that one co-twin was fed with more formula milk or expressed breastmilk) were examined (n=139) this association was statistically significant with a similar effect size to the previous comparison (Formula Feeding Discordance; d=-0.26, Broader IFM Discordance; d=-0.24). This indicates that the DT sample discordant for formula feeding may in fact have been underpowered to detect these meaningful differences. Moreover given the results generated under Aims 4.1 and 4.2, I conclude that maternal concern for low weight gain, may contribute to the parental decisions to introduce formula milk in the first weeks of life.

Whilst no previous studies, to our knowledge, have explored reciprocity towards concern for infant weight development, these findings align with findings from the UK Infant Feeding Survey[32], where concern for weight was reported as a key reason for the cessation of EBF. Secondly, we did not observe a significant difference in weight z-score at birth, 2 weeks, 6 weeks, nor in change z-score from birth to 3-months between co-twins discordant for formula feeding. Moreover, the supplementary DT comparisons with the larger IFM discordance group did not show significant differences. However, once again, a consistent trend whereby the co-twin fed with more formula milk was smaller at 2 and 6 weeks and gained less weight to 3-months of age was apparent and consistent with the reciprocity hypothesis. It is also important to consider that the smaller differences in weight change from birth to 3-months may be due to the influence of formula milk on growth after its introduction in the first weeks of life. As the previous analysis highlighted how formula milk can promote more weight gain relatively quickly, reciprocity towards growth may be masked in this specific DT comparison. However, we were unable to undertake this DT comparisons on change z-score prior to 3-months given the reduced number of DT twin pairs (n=62 pairs) with complete weight data prior to this time period. Nonetheless, we postulate that these trends with the findings of reciprocity under aim 4.1 and 4.2 of this study.

In sum, by triangulating numerous observational, longitudinal, and quasi experimental designs in Gemini - this study presented convincing evidence that formula milk introduction may, in part, be a behavioural response to slower weight gain or concern for low weight gain in early infancy. It is therefore important for future investigations and research to consider these bi-directional influences, particularly when making claims about cause and effect between feeding and growth. Where these bi-directional influences are unaccounted for, associations are may reflect unmeasured confounding from parent-child reciprocity in the relationship between feeding practices and infant growth.

4.5.3 Future Directions and Implications

4.5.3.1 Parent-Child Reciprocity in Infant Feeding; an Important Avenue for Research and Interventions

The present results highlight numerous important questions for future research and potential interventions to address. Firstly, these findings emphasize the need for future studies to account for reciprocity between feeding exposures and growth outcomes, both during after infancy. For instance, ensuring that infant feeding exposures (as an exposure) truly precede growth patterns (as an outcome) in observational studies. Where these bi-directional influences are unaccounted for, associations between IFMs and weight development are likely to reflect unmeasured confounding from parent-child reciprocity.

Second, I found that maternal concern for low weight gain between birth and 3-months of age lead to a higher likelihood of formula milk supplementation. This begs the question, are mothers reporting concern in response to clinically relevant weight faltering, or perhaps smaller decreases in weight? Future research would benefit exploring the correlation between parental reports of concern for low weight gain and the prevalence of clinically relevant weight faltering. If a strong correlation is present, encouraging parents to continue breastfeeding when they report concern about their child's weight gain, may lead to further weight faltering and harmful consequences for the child. Yet, if parental concern is often common without clinically relevant weight faltering, then alleviating concern and reassuring caregivers (where they wish to continue breastfeeding) may prove useful in supporting longer breastfeeding durations. Within the Gemini Baseline Sample – concern for low weight gain from 0-3 months (24.44% of infants had maternal reported concern for low weight gain) exceeded the presence of weight faltering at 3-months of age (15.41% of Gemini baseline sample experienced clinically significant weight faltering) suggesting that concern may not always occur in the presence of weight faltering. In line with this observation, it has been speculated that parental concern for adequate weight gain may be inadvertently exacerbated by the current UK-WHO growth standards. These curves standards represent the growth of EBF infants, who meet a strict set of demographic criteria[41,112]. As these UK-WHO infants may have been EBF during the first few months of life *because* they had higher early weight gain and no other major weight or health concerns, concern may be inflated within these growth references, leading to the increased supplementation with formula milk. In a similar fashion, using these growth references may lead HCPs to encourage formula milk supplementation to caregivers, as has been reported in qualitative reports.[152] However, these speculations must be tested to ensure supporting parents to alleviate concern does not result in unintended consequences for either mother or child. Moreover, it is important to place emphasis on the caregivers wishes in this process, as encouraging continued breastfeeding where it is not the desire of the caregiver may lead to negative emotions and stigma. Nonetheless, my results suggest that caregivers can modify IFMs in response to these concerns surrounding infant growth, and therefore they need be given greater consideration in future research, infant feeding guidance, and perhaps interventions.

Third, the present findings also suggest that 'one-size-fits all' approaches to infant feeding guidance, such as The UNICEF Baby Friendly Initiative[139] implemented to support breastfeeding, may be limited in their lacking consideration of important child-driven influences over IFMs. Hence, I propose that feeding support may be more motivating, engaging, and perhaps even effective if the dynamic 'two-way street' of infant feeding is acknowledged.[153] For instance, feeding polices that acknowledge the unique tensions or challenges that may arise in the mother-child feeding relationship (e.g. weight development or infant appetite) as not 'a fault' of the mother may result in longer durations of breastfeeding, or advantageous feeding behaviours. In a similar vein, such approaches might also help to reduce the commonplace negative emotions and stigmas that surround infant feeding and introduction of formula milk. For instance, a greater recognition of child-led drivers of formula milk supplementation might help to shift some of the 'blame' and stigma that parents feel when adopting formula feeding to support the growth of their child. Nonetheless, future research should test these postulations in practice, as well as seek to explore alternative child-led drivers of formula milk supplementation (e.g. infant appetite) to gain a more complete understanding of parent-child reciprocity in infant feeding. For instance, whilst infant growth may be a stronger predictor of formula supplementation in the early weeks of life where growth is a significant priority to caregivers – infant appetite traits that are expressed as infancy continues may be strong predictors of supplementation in later infancy. Finally, it remains important to still consider the consider numerous other determinants of feeding decisions – such as social, economic, and cultural influences – which also influence IFMs.[112] However, any theorical understanding of infant feeding needs to consider *both* infant-led *and* socioeconomic-led determinants of feeding. Yet, to date the spotlight has largely been on structural social, economic, and cultural drivers of infant feeding[43,112]. As the present findings point to additional and important child-led drivers of infant feeding decisions – parent-child reciprocity should also be considered in future theory, research and policy.

5.5.3.2 Formula Milk Supplementation: An Effective Strategy to Promote Weight Gain

Under Aim 4.2 of this chapter, I highlighted how infants introduced to formula milk showed more rapid growth than those who continued to be EBF (to ~3-months) to 12-months of age. Hence, the introduction of formula milk might indeed be an effective strategy used by parents to encourage weight gain. Future studies should explore therefore potential mechanisms which explain steeper weight gain following the introduction of formula milk. Mainstream hypothesis largely focusses on the programming of faster growth through nutritional differences between formula milk and breastmilk.[109,143] However, it also remains possible that non-responsive bottle-feeding may lead to overfeeding and therefore rapid weight gain.[50] This might be particularly apparent within this subset of infants who have been introduced to formula milk not from birth, but in response to slower weight gain or concern for weight gain. For instance, caregivers may be more likely to introduce formula feeding using more instrumental or pressuring feeding practices to help support their babies to gain weight where they are concerned. These behaviours may perhaps also form habitual un-responsive feeding practices, that carry on beyond the period of exclusive milk feeding. However, these speculations need to be tested in future studies as no studies to date have explicitly explored the interaction between PFPs and IFMs in their implications on infant weight development. Specifically, future studies could investigate whether certain PFPs – such as pressured feeding - might mediate or *explain* the relationships between formula milk supplementation and more rapid infant growth trajectories following its introduction. These findings could highlight promising behavioural targets for interventions seeking to reduce the prevalence of RIWG amongst infants fed with formula milk. Furthermore, if un-responsive feeding practices are overrepresented amongst infants introduced to formula in response to low weight gain or concern, as we have hypothesised, then supporting caregivers with alternative bottle-feeding practices might be particularly pertinent for these populations.

4.5.4 Merits and Limitations

First, this study benefits both from Gemini rich set of growth measurements and detailed measures of infant feeding practices. Specifically, as the timing (in days or weeks) of formula milk introduction was collected by mothers, this study is the first, to our knowledge, to disentangle growth trajectories *prior to* and *following* the introduction of formula milk. This helps to ensure feeding practices truly preceded growth outcomes - and visa-versa – in disentangling parent-child reciprocity. Whereas previous studies such as the CBGS and PROBIT, were merely able to compare growth trajectories in broad periods of early infancy such as 0-2 and 0-6 weeks in the CBGS.[70] However, whilst we were able to model growth trajectories surrounding the specific week of formula milk introduction for formula fed infants, for breastfed infants we modelled growth trajectories before and after 6-weeks of age. The 6-week point was chosen as the mean week of formula milk introduction, to facilitate these comparisons, however the more limited specificity of this measure for breastfed infants needs to be considered as well. This study also benefits from its use of the DT design. DT comparisons provide a unique opportunity to explore individual characteristics of twins which lead to discordant feeding – highlighting child-led determinants of infant feeding whilst removing confounding from shared environmental exposures that are shared by twins.[35,82] The overarching merits of using Gemini to explore reciprocity in infant feeding are further reflected on in Chapter Seven, Section 7.2.2.

However, this study is not without its limitations. Firstly, as aforementioned in Chapter Three, the growth of twins is not the same as that of singletons, given foetal restriction and a higher prevalence of catch-up growth.[21,22] For this reason, we excluded pre-term infants and adjusted for confounding influences such as time spent in specialist care, to help account for variations in anthropometric characteristics and improve the generalizability of the findings. Second, given the study design, we excluded infants who were introduced to formula in the first two weeks of life to allow for formula feeding to be a response to infant growth. Whilst this was necessary for the research question at hand, it led to a reduced sample size as 74.38% of the Gemini baseline sample fed with formula milk were introduced to formula in the first week of life. This led to a smaller sample (N=450), than might be available in a singleton sample. Hence, future cohorts with larger samples of infant who were weaned to formula feeding within the first few weeks of life, rather than from birth, would be useful to confer the results found in Gemini. Nonetheless, once again the collection of information on timing of formula milk introduction in Gemini is rare within other birth cohorts, and therefore enabled the present novel analyses to be undertaken.

Moreover, although the DT design offers a powerful tool to explore reciprocity, feeding decisions made in the context of caring for twins may vary from decisions made in the context of caring for singletons. Overall, the proportion of having ever breastfed was comparable between Gemini (77% of the sample) and nationwide data from the 2010 UK Infant feeding survey, at 69% for multiple infants.[52,142] However, breastfeeding two infants as opposed to one, might be more taxing and demanding for mothers, and mothers may be more concerned for adequate milk supply.[154] Moreover, slower weight gain in a twin may be more salient and result in more formula milk supplementation because a direct comparison can be made towards the weight development of a co-twin. Similarly, slower weight gain may be more concerning for a twin as they are born earlier and smaller, and are more likely to have health complications following birth. Despite these factors being adjusted for in the observational models, there may be a lower threshold for the introduction of formula milk towards slower weight gain in twins as compared to singletons. Whilst reciprocity has been measured in singletons in the smaller CBGS sample[70], it is worth bearing in mind that reciprocity towards slower growth may be heightened in a twin sample and should be replicated in singletons samples further.

Moreover, it is important to consider and reflect on the presence of expressed breastfeeding (through a bottle) within both EBF and formula fed infants in the current sample. These infants were included to increase the sample size and statistical power of the present models.

It is possible that the introduction of expressed breastfeeding might also be responsive towards slower infant growth in early infancy. Therefore, the presence of expressed breastfeeding feeding in both the EBF and mixed-and-formula fed groups may have diluted measured reciprocity between slower infant growth and formula milk introduction. To account for this, a supplementary analyses removing infants fed with expressed breastmilk from both the EBF and formula fed IFM groups was undertaken and conferred the results of the main models. Therefore, we propose that the introduction of formula milk may still be a response to slower infant growth independent of expressed breastfeeding. Moreover, whilst it would have been of merit to specifically measure whether the introduction of expressed breastfeeding is response to slower infant growth, Gemini did not measure the timing of introduction of expressed breastfeeding as it did formula feeding. Therefore, the present models under Aim 4.1 or 4.2 could not be replicated with expressed breastfeeding in this manner.

Finally, in relation to the second aim of the study, is also important to consider how infants may be more likely to demonstrate rapid weight gain when introduced to formula, if they experienced slower earlier growth or weight faltering. These infants therefore have more 'room-to-grow' and experience some degree of catch-up growth which contributes to faster growth trajectories following the introduction of formula milk.[40] Whilst this is important to consider, the current results demonstrated that infants supplemented with formula milk surpassed their EBF counterparts by 8 weeks following introduction, and substantially exceeded these EBF counterparts to 12-months of age. Hence, these results suggest that formula milk introduction may not only lead infants to 'catch up' in their weight development, but also continue on a steeper weight gain trajectory to 12-months of age. However, once again, future studies with singleton samples where catch-up growth is less prevalent, are well placed to confer the influence of formula feeding on infant growth specifically *after* its introduction where sufficient measures are available.

4.5.5 Concluding Statement

This chapter triangulated bi-directional longitudinal epidemiological analyses with the discordant twin (DT) design to explore parent-child reciprocity in infant feeding. Overall, our findings supported the reciprocity hypothesis. Hence, the introduction of formula milk may, in part, be a parental response to slower infant weight gain and concern for low weight gain in the early weeks of life.[40,70] DT comparisons conferred these results, suggesting that maternal concern for low weight gain contributes to discordant formula feeding across a twin pair. Hence, I would strongly urge future studies to consider parent-child reciprocity when measuring associations between feeding exposures and growth outcomes. Moreover, infant feeding interventions which consider parent-child reciprocity infant feeding decisions may be more effective in modifying feeding practices or supporting caregivers than commonplace ‘one-size fits all’ approaches. However, future studies should seek to explore how alternative aspects of infant feeding (e.g. PFPs) might also be responsive to infant growth, as well as other unique infant characteristics (e.g. appetite), to further disentangle the complexities of parent-child reciprocity in infant feeding. The second key finding of this study was that formula milk introduction increased rate of weight gain to 12-months of age, suggesting that formula milk supplementation may indeed be an effective strategy implemented by parents to promote weight gain. Future studies should therefore seek to explore potential mechanisms that explain how formula provision may promote RIIWG (e.g. bottle-feeding practices) to highlight targets for interventions. In sum, I conclude that both ‘parent to child’ and ‘child to parent’ directions of influence are important to consider in the relationship between infant feeding and infant growth.

Chapter Five. Study 3: Gene-Environment Interplay in Infant Feeding Modalities and Infant Weight Development

5.1 Introduction

Numerous twin studies demonstrate the early emergence of genetic influence on variations in weight development in infancy. The largest twin samples to date estimate heritability for weight z-scores in infancy to be approximately 29% at birth, increasing to 71% at 36-months of age.[94] Moreover, a more most recent and large systematic review (n= 87,782) estimating heritability of BMI across infancy and into childhood, measured heritability to lie at around 60% as early as 1-year of age.[85] However, similar to all complex phenotypes, infant weight development is likely to be a result of a complex interplay between environmental exposures and genetic influence – or GE interplay.[155] In line with this, there is an emerging hypothesis that IFMs may be responsive to early emerging infant characteristics such as slower weight gain[40,70], which may also be under genetic influence. In Chapter Four, I presented novel evidence that the introduction of formula milk, was responsive to slower early infant growth patterns. In this study, I will take this hypothesis a step further – exploring whether these same IFMs may be responsive to genetic influences over infant growth patterns – a **GE correlation**. Whilst no studies have explored GE correlations between parental feeding and child weight during the infancy period, a study in later childhood used genetically sensitive methods demonstrated that parental feeding practices were moderately heritable, with genetic influences explaining 43% and 54% of variation in restrictive feeding and pressured feeding respectively.[113] This suggests that PFPs are partly developed in *response* to genetically influenced characteristics of the child. In further support of this claim – the study used twin modelling to demonstrate that variations in PFPs were mediated, or explained, by genetic influences on child BM. Therefore, the authors concluded that PFPs in childhood are responsive to children's genetic liabilities.[113] However, no such studies have been undertaken as early as infancy. Therefore, the present study is the first to explore whether parental provision of formula milk is a response to an infant's genetic liability for slower weight gain in the first few weeks of life.

Surrounding GE interplay in infancy, there is also an emerging hypothesis that association between IFMs and RIWG demonstrated across observational cohorts may be contributed to by noise from GE interaction, as demonstrated in other relationships between breastfeeding and health outcomes.[156,157] In other words, IFMs may be associated with RIWG as they carry the potential to moderate genetic liability towards rapid weight gain, such that formula feeding allows for greater expression of genetic risk. Or visa-versa, exclusive breastfeeding could help protect infants from the expression of genetic risk of rapid weight gain. Few studies have explored such **GE interaction** in later childhood. Within the Gemini Twin study, the heritability of BMI at 4-years of age was nearly twice as high for children living in more obesogenic home environments (86%) as compared to children in less obesogenic home environments (39%).[119] However, only one genetically sensitive study to date has explored the potential for GE interaction in infancy. Using the UK ALSPAC cohort - Wu and colleagues found that EBF, versus formula feeding, modified the association between a genetic risk score for BMI and BMI trajectories to 20 years of age – supporting the presence of subtle GE interplay.[110] However, no studies to date have explored whether infant feeding exposures may modify genetic expression on weight gain in infancy itself. Hence, the present chapter is the first study to leverage the twin study to explore both GE interactions and GE correlations between IFMs and infant weight development. If GE interplay were to be detected in the present study, children at higher genetic risk may particularly benefit from interventions to support appropriate and responsive milk feeding practices. This is because these infants might be i) more likely to be weaned onto formula milk as opposed to being exclusively breastfed in response to their genetic liability (GE correlation) and ii) this provision of formula milk may maximise their genetic susceptibility towards faster weight gain in infancy (GE interaction).

5.2 Aims

The following chapter aims to explore potential GE interplay between IFMs and infant weight development during the first year of life. I will use the twin design to explore both novel GE correlation and GE interaction between IFMs and infant weight gain.

To test GE correlation, I seek to test the hypothesis that caregivers may introduce formula milk in *response* to their infant's genetic liability towards weight gain in early infancy. I will undertake a two-stage approach. First, I will quantify genetic and environmental influences on IFMs during the first 3 months of life using a classical twin model. If IFMs show significant heritability, this would suggest that IFMs are partly driven by genetically influenced characteristics of the infant (e.g. weight). If such heritability is detected, I will then undertake a second stage of analyses to investigate whether genetic influence on IFMs are mediated by genetic influence on rate of infant weight gain from 0-3 months of age. This will test the hypothesis that IFMs are, in part, a response to genetic influence on infant weight gain, during the earliest period of postnatal life. Given the presence of reciprocity between IFMs and infant weight patterning in Chapter 4, I hypothesise that: (i) variation in IFMs at 3-months of age is under significant genetic influence; and (ii) genetic influence on variation in IFMs is mediated by genetic influence on variation in infant weight gain.

The second aim was to explore GE Interaction by testing the hypothesis that parental provision of formula milk provides greater propensity for expression of genetic liability towards RIWG in the first year of life. I will utilise a heterogeneity (GE interaction) twin model, which tests whether genetic, shared environmental and non-shared environmental influences on variation in infant weight gain differ according to infant feeding exposures. Higher heritability estimates for rate of infant weight gain for formula-fed than breastfed infants would indicate that formula feeding maximises genetic liability to rapid infant weight gain. Given the demonstrated presence of GE interactions at 4 years of age in Gemini[119], and within the single previous GE interaction study of IFMs[110], I hypothesised that the heritability of infant weight gain from 0-12 months of age would be significantly higher for formula fed (and perhaps mixed fed) infants than breastfed infants.

Aim 5.1: Explore Gene-Environment Correlation in IFMs and Weight Development

RQ 1: Quantify the contribution of additive genetic variance (A), shared environmental factors (C), and non-shared environmental factors (E) to variation in Infant Feeding Modalities (IFMs) during the first ~3 months of life

- ➔ *Hypothesis: Additive genetic variance will contribute moderately to variation in IFMs at ~3 months of age*

RQ 2: Explore whether genetic influence on variation in IFMs at ~3 months of age is mediated by genetic influences on variation RIWG from 0-3 months of age

- ➔ *Hypothesis: A significant proportion of genetic factors will be shared between IFMs at ~3-months of age and RIWG* from 0-3 months of age

Aim 5.2: Explore Gene-Environment Interaction in IFMs and Weight Development

RQ 3: Explore whether the contribution of additive genetic variance (A), shared environmental factors (C), and non-shared environmental factors (E) to variation in RIWG from 0-12-months of age differs across IFMs (exclusively breast, mixed and formula fed infants)

- ➔ *Hypothesis: the heritability of RIWG from 0-12-months of age will be higher for formula fed and mixed-fed infants than exclusively breastfed infants.*

5.2 Methods

5.2.1 Samples

For this chapter, I used data from the Gemini baseline questionnaire collected at ~8 months and the second wave of data collection at 16-months and of age, described in Section 2.3.1 of Chapter Two.

5.2.1.1 Aim 5.1: Explore Gene-Environment Correlation in Infant Feeding Modalities and Weight Development

The sample of eligible infants for the univariate model of IFMs (at ~3-months of age) consisted of 2151 twin pairs (4,302 twins) with complete data on the IFMs and valid information on zygosity reported at baseline. I did not undertake a BT model examining genetic overlap between infant feeding modality and infant weight gain because the results of the univariate twin model did not support moving forwards with this analysis. The methods or samples for this second stage have therefore not been outlined here. Pre-term infants (<36 weeks) were not excluded in the present analyses to increase the power necessary of univariate twin models.

5.2.1.2 Aim 5.2: Explore Gene-Environment Interaction in Infant Feeding Modalities and Weight Development

The Heterogeneity (GE Interaction) Twin Model included n=2151 twin pairs (n=4302) with reported zygosity, as well as the complete data for the moderator term (IFMs in the first 3-months of age). Pre-term infants (<36 weeks) were not excluded in the present analyses to increase the power of the GE Interaction twin models.

5.2.2 Measures

5.2.2.1 Infant Feeding Modalities

Information on infant feeding methods was collected at baseline when infants were ~8-months of age (M=8.2 SD=±2.2 months). The methods and resulting IFM groups are describe in Chapter Two Section 2.2. From these responses, an IFMs measure was generated to represent three groups: EBF infants; mixed-fed infants; and primarily formula fed infants. A broader 3-category measure was chosen over the more detailed 7-category measure to maximise statistical power, as twin models require large samples for genetic and environmental components of variance to be estimated reliably. These groups are described and presented in Figure 2.2 of Chapter Two. Overall, n=4302 (89.50%) infants (n=2151 pairs) with complete zygosity had IFM sufficient data to be included in this measure: n=634 (14.73%) were categorized as 'exclusively breastfed'; n=2071 (48.14%) were categorized as 'mixed fed'; n=1,597 (37.71%) were categorized as 'formula fed'. Overall, n=401 infants had missing information on IFMs and n=101 infants had missing information on zygosity and could not be included in the analysis sample.

5.2.2.2 Rapid Infant Weight Gain from 0-3 and 0-12 months of age

From 8-months of age, parents were asked to copy the weight measurements and dates from their personal child health records into the relevant questionnaire for each twin. These weight measurements, alongside the infant's gestational age (in weeks) and sex were used to calculate weight-for-age z-scores at birth and 3- and 12-months of age using the UK-WHO Growth Standards in the zAnthro package in Stata[126]. Change in weight-for age z-score was calculated by subtracting birth z-score from the 3- or 12-month z-score. Change in weight for age z-score from 0-3 months was intended to be used as a continuous outcome measure for the BT Model of Infant Feeding Modality and Infant Weight Gain under Aim 5.1. Whilst change in weight for age z-score from 0-12 months was used as a continuous outcome measure for the heterogeneity (GE interaction) twin model under Aim 5.2. The clinical binary measure of RIWG (i.e. rapid weight gain versus no rapid weight gain) was not used in either analysis, in order to maximise power for the heterogeneity (GE interaction) twin model.

5.2.2.3 Zygosity

For details of zygosity of the twins was established and validated against genomic data see section 2.5.2 of Chapter Two.

5.2.3 Statistical Analysis

5.2.3.1 Aim 5.1: Explore Gene-Environment Correlation in Infant Feeding Modalities and Weight Development

For the first aim, I sought to explore genetic and environmental influences on IFMs within the first ~3 months of life. The twin design allows for the estimation of genetic and environmental contributions to variation in a trait of interest by comparing MZ twin pairs (who share 100% of their genetic material) to DZ twin pairs (who share ~50% of their segregating genes). As both twin pairs share their environments to a very similar extent, particularly in the infancy period, a greater similarity in IFMs between MZ pairs as compared to DZ pairs would suggest a genetic contribution to variation in IFMs. For a more detailed explanation of the methods used to measure genetic and environmental influences under the twin design see Section 2.5.3.

The first step is to produce a contingency table of IFM groups (EBF, mixed fed, formula fed) to examine concordance across MZ and DZ twin pairs. The pattern of concordance across MZ and DZ pairs provides an indication of the extent of genetic, shared environmental and unique environmental influence underlying variation in IFMs. Specifically, greater concordance between MZ than DZ pairs indicates a genetic contribution to variation in IFMs. However, little or no difference between MZ and DZ concordance in IFM indicated little or no genetic contribution to variation in IFMs. Rather, this pattern of concordance indicates that IFMs are largely determined by environmental factors entirely shared by co-twins (e.g. socioeconomic position).

Twin pair polychoric correlations for IFMs were then calculated for MZ and DZ twins using the OpenMX package, Version 2.2.5, in R.[136] If MZ pairs were more concordant for IFM than DZ pairs, then a MLSEM would be used to provide more precise estimates of additive genetic influence (A) (as well as shared (C) and non-shared environmental influence (E), with 95% CIs, and goodness-of-fit statistics (e.g. AIC and Likelihood Ratio Test (LRT) to indicate the most parsimonious model fit. Statistical significance was set at $p<0.05$, and p-values were 1-sided for the intended MLSEM models.

The next step of the analyses was intended to be a BT twin model measuring whether genetic influences underlying variation in IFMs at 3-months of age and genetic influences over RIWG at 12-months of age may be shared. However, these methods are not detailed as this model could not be undertaken following the results of the Univariate Model of IFM.

5.2.3.2 Aim 5.2: Explore Gene-Environment Interaction in Infant Feeding Modalities and Weight Development

The univariate ACE Model described above can also be extended to generate a heterogeneity (GE interaction) univariate ACE model, to test for differences in the magnitude of A, C and E estimates for variation in infant weight gain from 0-12-months, between IFM groups (breastfed, mixed fed and formula fed). This model allows A, C, and E parameter estimates to differ across groups, following the same stages as described above. For a detailed description of this model see Section 2.5.5 of Chapter Two.

Twin Correlations and Model Fitting

Once again, two methods were used to estimate the heritability of change in weight for age z-score from 0-12-months of age between the three IFM groups: i) twin correlations and ii) MLSEM. First, twin pair correlations were calculated for MZs and DZs, stratified by each IFM group (EBF, mixed-fed, formula fed) using structural equation modelling software in OpenMx. Then, a heterogeneity (interaction) twin model (called a 'common effects model') was used to test for differences in parameter estimates (A, C, and E) across the three IFM groups. The common effects model was then compared to a more constrained scalar model, which contains the variance to be the same across groups, and then a final null model, which constrains both the variances and the parameter estimates (A,C and E) to be the same across groups. Where the null model (no GE model) shows no significant reduction in fit as compared to the common effects model (GE model), this would indicate that there are no differences in the magnitude of the parameter estimates for A, C or E across the IFMs groups. 95% CIs for the parameter estimates and goodness-of-fit statistics will be presented. The heterogeneity model was generated in R using the structural equation modelling software OpenMx, version 2.2.6. Statistical significance was set at $p<0.05$.

5.3 Results

5.3.1 Sample Characteristics

The sample characteristics for the Heterogeneity MSLEM (GE interaction) model for RIWG from 0-12 months (Aim 5.2) are presented alongside the characteristics of the Baseline Gemini Sample in **Table 5.1** below. The GE Interaction sample showed similar distributions to the Baseline sample of Gemini twins across; Zygosity, feeding modalities, sex discordance, party, maternal BMI, mode of delivery, as well as measures of socioeconomic positioning such as NS-SEC and Index of multiple deprivation. The sample characteristics for the univariate MSLEM model on IFMs (Aim 5.1) are not presented as this model could not be undertaken due to the high twin pair concordance in IFMs measured at ~3-months of age.

Table 5.1. Descriptive Statistics of Baseline Sample of Gemini Twin Pairs Compared to Gemini Twins Included in GE Interaction and GE Correlation Models

		Baseline Sample of Gemini Twin Pairs (n=2404)		Sample of Gemini Twins Included in GE Interaction Model and GE Correlation Samples (n=2151)	
		N	Mean (SD) or %	*N*	Mean (SD) or %
Infant & Familial Characteristics					
Zygosity					
	Dizygotic	1,616	68.33	1,460	67.87
	Monozygotic	749	31.67	691	32.12
Infant Feeding Modality[a]					
	Breastfed Breastfed	417	19.13	413	19.20
	Mixed Fed Mixed Fed	883	40.50	874	40.63
	Formula Fed Formula Fed	796	36.51	783	36.40
	Breastfed Mixed Fed	57	2.61	55	2.56
	Breastfed Formula Fed	4	0.18	4	0.19
	Mixed Fed Formula Fed	23	1.06	22	1.02
Sex Discordance					
	Male, Male	785	32.68	703	32.49
	Female Female	801	33.35	718	33.18
	Male, Female	816	33.97	730	34.33
Ethnicity					
	White	2,231	92.96	2,012	93.02
	Other	169	7.04	151	6.98

BMI (mother)	2,338	25.10 (4.75)	2,108	25.06 (4.73)
Gestational Age (in weeks)	2,392	36.20 (2.48)	2,151	36.31 (2.34)
Mode of Delivery				
Caesarean	1,559	65.20	1,409	65.41
Vaginal	832	34.80	745	34.59
Parity				
None	1,199	51.42	1,060	50.50
One or More	1,133	48.58	1,039	49.50
National Statistics Socioeconomic Class (NS-SEC)[b]				
High	1,515	63.28	1,382	64.07
Middle	470	17.00	359	16.64
Lower	472	19.72	416	19.29
Index of Multiple Deprivation[c]				
Least Deprived	613	25.78	550	25.69
Not Deprived	573	24.10	516	24.10
Mid	476	20.02	427	19.94
Deprived	412	17.33	374	17.47
Most Deprived	304	12.78	274	12.80

*** p < 0.001; ** p < 0.01; * p < 0.05.

[a]Exclusively Breastfed: Fed breast milk from the breast or from expressed milk; Expressed Milk Fed; Fed with primarily expressed milk from a bottle or equal amount of breastmilk from the breast and expressed milk from a bottle; Mixed-Fed: Fed with a combination of breast milk from the breast, expressed milk in a bottle, and formula milk; Formula Fed: Fed with formula milk
[a]Household NS-SEC based on the Higher Ranking Position of the two full NS-SECs
[c]5 quintiles of deprivation, 1 = 'score ≤8.49 (least deprived quintile)', 2 = '8.5–13.79', 3 = '13.8–21.35', 4 = '21.36–34.17', 5 = '≥ 34.18 (most deprived quintile)'
% for analytic sample do not include missing data to represent distribution of variables across infants included in the sample

5.3.2 Aim 5.1: Gene-Environment Correlation in Infant Feeding Modalities and Weight Development

5.3.2.1 Twin Correlations in Infant Feeding Modalities

Contingency tables of IFM groups (EBF, mixed fed, formula fed) across MZ and DZ twin pairs are presented in **Tables 5.2 and 5.3.** These tables show concordance and discordance rates in IFMs across both twin types to explore whether genetic influences were likely to contribute to variation in parental feeding. Of the 688 MZ pairs and 1463 DZ pairs with complete IFM data, a very small number of pairs were discordant for IFMs in either MZ (n=10, 1.5% of MZ pairs) or DZ (n=48, 3.3% of DZ pairs) pairs. In line with this, the twin pair correlations were very high for both MZ (b=0.99) and DZ (b=0.98) pairs, showing that co-twin variation was rare regardless of zygosity (See **Table 5.2**). Such low variation indicated no genetic contribution to IFMs, and meant it was not possible to move forward with the univariate ACE models, derived using MLSEM. Therefore, the final stage of analysis (a BT of shared genetic variation between IFMs and infant weight gain) was also not undertaken.

Table 5.2. Contingency Table of IFMs at 3-months Across Monozygotic and Dizygotic Twin Pairs

Infant Feeding Modalities (Ordinal; Exclusively Breastfed, Mixed-Fed, Formula Fed)								
		Monozygotic Twin Comparisons (n=688 pairs)				Dizygotic Twin Comparisons (n=1463 pairs)		
		Exclusively Breastfed	Mixed fed	Formula Fed		Exclusively Breastfed	Mixed fed	Formula Fed
	Exclusively Breastfed	98 (14%)	5 (1%)	0 (0%)		204 (14%)	16 (1%)	0 (0%)
	Mixed fed	0 (0%)	318 (46%)	3 (0.6%)		11 (0.7%)	690 (47%)	12 (0.8%)
	Formula Fed	0 (0%)	2.(0.3%)	262 (38%)		2 (0.1%)	7 (0.5%)	521 (37%)

IFM; 3 Groups (i) Exclusively Breastfed from the breast or expression ii) Mixed fed with breastfeeding, expressed feeding and formula feeding iii) Predominantly formula fed

Table 5.3. Variance-covariance matrix showing polychoric correlations for IFMs between MZ and DZ pairs

Infant Feeding Modalities (Exclusively Breastfed; Mixed-Fed; Formula Fed)					
		Monozygotic Twin Comparisons (n=688)		Dizygotic Twin Comparisons (n=1463)	
		Twin 1	Twin 2	Twin 1	Twin 2
	Twin 1	1.0000	0.99	1.0000	0.98

IFM; 3 Groups (i) Exclusively Breastfed from the breast or expression ii) Mixed fed with breastfeeding, expressed feeding and formula feeding iii) Predominantly formula fed

5.3.3 Aim 5.2: Explore Gene-Environment Interaction in Infant Feeding Modalities and Weight Development

5.3.3.1 Twin Correlations

For change z-score from 0-12-months, correlations were substantially higher between MZ than DZ twins across all IFM groups (range; 0.85-0.82 vs 0.67-0.77), suggesting an additive genetic contribution to variation in infant weight gain during the first year of life. The magnitude of the difference between MZ and DZ twin pair correlations varied slightly across IFMs, with larger differences between MZ and DZ pairs in mixed-fed infants (MZ; 0.85 vs. DZ; 0.67, Difference; 0.18) and formula fed (MZ; 0.85 vs. DZ; 0.71, Difference; 0.14) infants, as compared to EBF Infants (MZ; 0.82 vs. DZ; 0.76, Difference; 0.06). As subtle differences in MZ and DZ correlations were highlighted, I undertook a heterogeneity (interaction) ACE model using MSLEM.

Table 5.4. Intraclass Correlations of Change in Weight for Age Z-Score at 12- months by Zygosity and IFM Group

Change in weight-for-age z-score from birth to 12-months					
Exclusively Breastfed Infants (n=317)					
		Monozygotic Twin Comparisons		Dizygotic Twin Comparisons	
		Twin 1	Twin 2	Twin 1	Twin 2
	Twin 1	1.0000	0.8282793	1.0000	0.7664039
Mixed Fed Infants (n=1036)					
		Monozygotic Twin Comparisons		Dizygotic Twin Comparisons	
		Twin 1	Twin 2	Twin 1	Twin 2
	Twin 1	1.0000	0.8530775	1.0000	0.6686179
Formula Fed Infants (n=798)					
		Monozygotic Twin Comparisons		Dizygotic Twin Comparisons	
		Twin 1	Twin 2	Twin 1	Twin 2
	Twin 1	1.0000	0.8479923	1.0000	0.7085992

Weight-for Age Z-Scores: Calculated using UK-WHO growth refence data adjusting for age, sex, and gestational age.

IFM; 3 Groups (i) Exclusively Breastfed from the breast or expression ii) Mixed fed with breastfeeding, expressed feeding and formula feeding iii) Predominantly formula fed

5.3.3.2 Maximum Likelihood Structural Equation Modelling

For 12-month change z-score, the null and scalar models (No GE interaction Model) were not a significantly worse fit to the data than the common effects model (GE interaction model; n=2151) (full fit statistics reported in Appendix IX) suggesting that the ACE components did not vary significantly across IFM groups. The parameter estimates of the No GE ACE model, alongside the GE model, are summarized **Table 5.5**. Across the total sample, variance in change z-score at 12-months was largely attributable to shared environmental factors (54%; 95% CI, 46%-60%), moderately attributable to additive genetic factors (32%; 95% CI, 24%-40%), and nonshared environmental factors (14%; 95% CI, 12%-17%).

Table 5.5. Parameter Estimates and Goodness-of-Fit Statistics for GE Interaction Models Examining Heritability of Change Z-Score from 0-12-months by IFMs (n=2151 pairs)

			Change in Weight for age z-score from 0-12 months					
			Estimate[a]					
			Additive Genetic	Shared Environment	Non-Shared Environment		Change in AIC	P-Value
GE Model (Common Effects Model)								
	Exclusively Breastfed		0.26 (0.11, 0.44)	0.58 (0.41, 0.71)	0.16 (0.11, 23)		-	-
	Mixed Fed		0.32 (0.21, 0.45)	0.53 (0.42, 0.64)	0.15 (0.11, 0.18)		-	-
	Formula Fed		0.39 (0.25, 0.54)	0.47 (0.33, 0.59)	0.14 (0.11, 0.19)		-	-
No GE (Null Model)			0.32 (0.24, 0.40)	0.54 (0.46, 0.60)	0.14 (0.12, 0.17)		*4.612*	*0.7083*

[a]Estimates represent the proportion (in %) of variation in Change Z-score that is due to additive genetic effects (A), shared environmental effects (C), and unique or non-shared environmental influences (E)

Weight-for Age Z-Scores: Calculated using UK-WHO growth refence data adjusting for age, sex, and gestational age.

IFM; 3 Groups (i) Exclusively Breastfed from the breast or expression ii) Mixed fed with breastfeeding, expressed feeding and formula feeding iii) Predominantly formula fed

5.4 Discussion

5.4.1 Summary of Findings

The present study is the first, to my knowledge, to test for the presence of both GE correlation and GE interaction in the relationship between IFMs and infant weight development. To explore GE correlation, I sought to quantify the contribution of additive genetic variance (A), shared environmental factors (C), and non-shared environmental factors (E) to variation in IFMs used within the first ~3 months of life. I hypothesized that additive genetic variance would contribute moderately to variation in IFMs, suggesting that IFMs may be a response to a child's genetic predisposition to slower weight gain, in line with the findings from Chapter Three. However, very high concordance rates between both MZ and DZ twins, were indicative of very little genetic influence over variation in IFMs – suggestive of no GE correlation. Taken together, these findings suggest that IFMs used in early infancy may be more strongly influenced by i) social and economic factors[43,112] and ii) early fluctuations in weight gain[40,70] or concern for weight gain (as demonstrated in Chapter Three), as opposed to the genetic liability over early weight gain itself.

Second, I sought to explore GE interaction by investigating whether the contribution of additive genetic variance (A), shared environmental factors (C), and non-shared environmental factors (E) to variation in RIWG from 0-12-months of age varied across breastfed, mixed-fed and formula fed infants. I hypothesised that the heritability of RIWG at 12-months would be higher amongst formula fed infants and mixed-fed infants, as compared to EBF infants, as formula feeding might provide a greater opportunity for the genetic liability towards RIWG to express itself across the first year of life – a GE interactio*n*. Overall, the heterogeneity univariate twin model indicated that genetic and environmental influences over RIWG did not vary significantly across IFM groups. However, we observed a non-significant pattern whereby formula fed infants and mixed fed infants showed a trend towards higher heritability for RIWG at 12-months, as compared to EBF infants. Given these patterns, it would be valuable to continue investigating GE interactions in infant feeding with alternative twin cohorts, as ours is the first investigation on this subject. Nonetheless, my findings did not generate strong support the presence of GE interplay between IFMs and infant weight development in Gemini.

5.4.2 Comparison of Findings to Previous Literature

5.4.2.1 Aim 5.1: Explore Gene-Environment Correlation in Infant Feeding Modalities and Weight Development

Firstly, contrary to my hypothesis, IFMs reported in the first 3-months of life did not appear to be responsive to genetic liability towards slower infant weight gain from 0-3 months of age (a GE correlation). Within the Gemini Twin sample, twin pair concordance for IFMs in the first ~3-months of life was very high for both MZ and DZ twin pairs which suggests that there was no genetic contribution to variation in IFMs. This would suggest that IFMs in early infancy are not a behavioural response to genetic liability for infant weight development. One explanation may be that such reciprocity may not emerge so early in infancy where the heritability of weight has been shown to be lower (38% at 3-months in Gemini) than in later infancy (62% at six months in Gemini).[91] If early weight development is under less influence from genetic factors – albeit there is still some measured genetic influence at 3-months – than reciprocity towards slow weight gain may not represent underlying genetic influences. Hence, IFMs used in early infancy may more strongly influenced social and economic factors, as demonstrated by the substantial contribution of shared environmental influences to RIWG from 0-12 months in Gemini (54%). Similarly, as demonstrated in Chapter Three, IFMs may also be under greater influence from fluctuations in infant weight gain or perceptions of infant weight gain, than genetic influences over early infant weight gain itself. Whilst no studies have explored GE correlation in infant feeding, apart from ours, studies from later childhood have begun to demonstrate the presence of GE correlation in toddlerhood and childhood. For instance, in a similar analyses of the TEDs Twin Study at 10-years of age – restricting and pressuring PFPs were both moderately to highly heritable.[113] Moreover, there was a genetic correlation between higher child BMI and higher parental restriction, and lower child BMI and higher parental pressure – suggesting that parental feeding behaviour may be influenced by genetic influences over child BMI.[113] Whilst these findings might suggest that alternative aspects of infant feeding to IFMs – such as PFPs or bottle-feeding practice – might be more responsive to genetic liability over weight development. However, once again, no previous studies have explored these GE correlations in infant feeding and thus future twin studies are needed to corroborate the present findings and explore alternative aspects of potential GE correlation.

5.4.2.2 Aim 5.2: Explore Gene-Environment Interaction in Infant Feeding Modalities and Weight Development

In regard to GE interaction, I hypothesized that heritability estimates for RIWG (at 12-months of age) would be significantly higher amongst formula fed and mixed fed infants, as compared to EBF infants. This would suggest that formula feeding offers more opportunities the *expression* of genetic risk towards rapid growth. However, I did not find significant differences in the heritability estimates for RIWG from 0-12 months across infant feeding modalities. Therefore, the ability for feeding methods and behaviours to moderate the expression of genetic risk for weight development might emerge in later in childhood or with alternative aspects of feeding.[119] Moreover it remains possible that alternative aspects of infant feeding -such as restrictive or pressuring feeding – might moderate genetic liability towards infant weight development which has yet to be examined. However, it is worth noting that I observed an insignificant trend towards higher heritability estimates for RIWG at 12-months of age in formula fed infants (A= 0.39) as compared to EBF infants (A=0.26), which the current sample may not have been powered to detect. Hence, larger twin samples with measures of IFMs are warranted to confer the present results. Moreover, using polygenic indices might offer more another useful design to explore GE interactions in addition to the twin design. In one such previous GE interaction study, Wu et al (n=5,266) found that EBF to 5-months had a stronger protective effect on BMI at 18-years of age for singletons with a higher PRS as compared to lower PRS.[110] However, when looking at weight trajectories in infancy itself, less marked GE interaction was observed. EBF delayed the age of adiposity peak (an advantageous growth pattern) for boys of a higher PRS more so than boys of a lower PRS. Yet, no GE was present in relation to adiposity rebound. Whilst our results are not directly comparable as i) Wu and colleagues did not measure RIWG and ii) they used a measure of EBF to 5-months as opposed to 3 months, which might offer a longer opportunity to buffer genetic risk, they are useful to consider as the only previous study to consider GE interaction within infancy. Moreover, in relation to GE interactions in childhood, previous findings from Gemini have demonstrated that heritability of BMI at 4-

years of age was nearly twice as high for children living in more obesogenic home environments (86%) as compared to children in less obesogenic home environments (39%).119 This suggests a modification of genetic expression on weight might depend on wider socioeconomic exposures, as opposed to more proximal feeding exposures. Such GE interactions would also be of merit to investigate as early as infancy. Hence, I conclude that whilst the present study found no convincing evidence of GE interaction in infancy – further studies with alternative aspects of infant feeding or environmental exposures are warranted given the scarcity of research on GE interplay in infancy.

5.4.3 Future Directions and Implications

Several opportunities for future studies arose from the current results. First, the current analysis sought to explore GE interaction and correlation in regard to a broad measure of IFMS - EBF, mixed-fed and formula fed - which mothers self-reported when infants we're ~3-months of age. This variable was chosen over the more detailed 7-group IFM variable (derived under Study 1) as it would have segregated infants into too small of groups to power the intended twin models. Hence, this 3-group measure may in fact have been too crude to detect ways in which parents may adapt feeding to the genetic liabilities of their child. Moreover, Wu et al's measure of EBF measured to 5-months, may be able to show stronger GE interactions given the longer duration of breastfeeding to buffer expression of genetic risk.110 In a similar vein, alternative aspects of infant feeding – such as the volume of milk offered to children or PFPs may also be responsive to genetic liability, which was not explored in this study. As GE interactions have been demonstrated between PFPs and weight development in later childhood113,114, examining GE interplay with different aspects of feeding in infancy may be a valuable avenue for future studies. However, given the novelty of the current study, I also urge future studies to continue to consider GE interplay in relation to IFMs.

Secondly, using genetically sensitive approaches alternative to the twin design would be valuable in exploring GE interplay in singleton cohorts where twin comparisons are not possible.35 One such approach involves the utilisation of polygenic indices of genetic risk towards a phenotype – such as a polygenic risk score (PRS).158 Complex phenotypes, occur as a result of many genomic variations - and when such variants which are related to the phenotype of interest are identified - they can be combined to derive a marker of an individual's 'poly' genetic susceptibility towards that phenotype. PRS could be utilised to explore GE interplay within infancy in numerous ways. For example, one could explore whether the association between PRS for RIWG and infant weight gain – is stronger amongst formula fed, as compared to breastfed infants. However, as a PRS score does not currently exist for infant weight gain or RIWG, it is only possible to use PRS towards later weight development in such studies. For example, using this approach, Wu and colleagues demonstrated that EBF to 5 months delayed the timing of adiposity peak more so in boys of a higher adult PRS for than a lower adult PRS.110 Whilst the availability and affordability of genotyping is increasing, it remains both costly and ethically challenging to implement in early childhood. However, we postulate that once a PRS for weight gain within infancy becomes available, it should be applied alongside the twin design to further explore potential GE correlation and interactions in infant feeding. Moreover, polygenic data could also offer novel study designs to explore GE interplay. For example, Mendelian randomisation can be used as a natural experiment to explore GE interplay such as within Herle and colleagues TEDs analysis in later childhood.132

In regard to theoretical avenues for further research it would be of enormous value to explore the role of appetite in potential GE interactions between infant feeding and infant weight. A child's unique appetite has been postulated to play a mediating role in these relationships – such that genetic liability may be more strongly associated with resulting feeding practices where children express certain appetitive traits. For instance, in RESONANCE, Jansen and colleagues (n=197) found that the relationship between a child's BMI PRS and restrictive feeding was moderated by child's food responsiveness.[114] Parents therefore used more restrictive feeding practices in response to higher genetic risk for BMI when the child showed a more avid appetite, as compared to a less avid appetite. Whilst this finding related to appetite traits measures at 18-months, the moderating role of appetite may also play an important role in infant feeding. For instance, perhaps the use of formula feeding in response to genetic liability over weight would be stronger for babies of a larger appetite, who might be more demanding for milk and mothers may feel they are not satiating their infant with only breastmilk. However, these speculations need to be tested in further research.

5.4.4 Merits and Limitations

The present findings should be interpreted in light of several merits and limitations, particularly given their novelty.

Firstly, the novel study benefits from the concurrent exploration of both GE interaction (how genetic expression responds to the environment) and GE correlation (how the environment responds to genetic susceptibility). This allows for a more nuanced and "complete" understanding of GE interplay, which might help shape public health interventions and policies that consider the role of gene-environment interactions in future. Moreover, as discussed in greater detail in Chapter Seven, Section 7.2.2, the current study benefits from Gemini's nuanced measures of IFMs and health-professional collected measures of infant weight gain.

Nonetheless, given the present study utilised twin data to explore GE interplay, inherent limitations of the twin design must be considered. Twin modelling relies on the assumptions that MZ and DZ twins share their home and social environments to the same extent (so-called the equal environments assumption (EEA)).[159] However, MZ twins may be treated more similarly, or select into more similar environments, than DZ twins. If this environmental difference increases similarity between MZ twins, the result is inflated heritability estimates (which are estimated by modelling the difference between MZ and DZ similarity). However, as we did not demonstrate significant heritability of IFMs (GE correlation) or variations in the heritability of RIWG (GE interaction) across IFMs the risk of this bias is minimal. Moreover, it is worth reflection on how the current twin sample was of a high SES (61.84%) and was largely white (92.96%). Given that some twin studies present higher genetic estimates for BMI among white adolescents as compared to those from an east Asian family background[95,133], these parameter estimates should be replicated in a more diverse sample.

Secondly, in relation to the GE interaction analysis, it is important to consider how the growth of twins differs from the growth patterns of singletons.[21] As aforementioned in this thesis, the Gemini twins showed high rates of RIWG at 12-months of age (79.24%). Hence, the environmental and genetic influences on RIWG from 0-12 months of age should be extrapolated to singleton populations with caution. However, this limitation is somewhat inevitable as the heritability of RIWG can only be estimated through twin pair comparisons, or perhaps using polygenic scores of infant weight gain which are not currently developed or available. Moreover, there is no evidence that growth patterns differ systematically between MZ and DZ pairs[91], which would compromise twin pair comparisons of infant growth.

Finally, it is important to consider how EBF infants captured by the measure of IFMs used consisted of both infants breastfed from the breastfed and from expressed breastmilk into a bottle. One proposed mechanism for formula feeding allowing for a greater expression of genetic influences on weight development is that bottle-feeding may allow for greater speed or volume at which infants can feed from a bottle.[50,160] This more rapid consumption of milk may in turn inhibit regulation of hunger and satiety cues when repeated regularly.[161] Whilst this mechanism remains largely untested, if expressed breastfeeding allows for the expression of genetic risk, the presence of expressed feeders in the EBF may have diluted the present heterogeneity model results by increasing the similarity of heritability estimates between the feeding groups. Hence, a stricter comparison of infants fed from the breast versus from formula milk may have shown starker variations in heritability of RIWG. However, breastfed infants exclusively fed through expression into a bottle were a very small subset of EBF infants (n=66, 10% of EBF infants) and therefore the influence of this limitation is likely to be small. Moreover, significant GE interplay was not identified in the current model, and therefore we deemed it unnecessary to run a supplementary analysis excluding expressed feeders to account for this limitation. Nonetheless, it is important for future studies to carefully consider how IFMs are being measured and represented when making comparisons between ‘breastfed’ and ‘formula fed’ infants.

5.4.5 Concluding Statement

The present study was the first, to our knowledge, to explore both GE correlation and GE interaction in the relationship between IFMs and infant weight development using the twin design. In Gemini, neither significant GE interaction nor GE correlation was measured. Hence, IFMs reported in the first 3-months of life did not appear to be responsive to genetic liability towards slower infant weight gain from 0-3 months of age (a GE correlation). Twin pair concordance was high for IFMs in Gemini suggesting no genetic contribution to variation in IFMs. Hence, early IFMs may be more strongly driven by social and economic factors, or fluctuations in infant weight gain and perceptions of infant weight gain (as highlighted in studies 1 and 2). It may also be the case that reciprocity towards genetic influences over weight begins to emerge in later childhood[113,114], although more studies with alternative measures of infant feeding- such as PFPs - are warranted. Second, contrary to my hypothesis, I did not find that the heritability of RIWG at 12-months of age was significantly higher amongst infants fed with formula milk than those EBF (a GE interaction). However, I postulate that the current twin model might have been underpowered to detect subtle differences in the heritability of RIWG, as heritability estimates were slightly higher for formula fed and mixed-fed infants as compared to EBF infants in line with the hypothesis. Hence, alternative twin investigations are needed to corroborate the lack of GE interactions found in the current Gemini analyses. Given the novelty of the current study, I urge future research to continue to explore GE interplay in relation to both IFMs and alternative aspects of infant feeding and infant weight development.

Chapter Six: A Digital Intervention to Promote Responsive Formula-Feeding and Healthy Growth in Infancy; a Protocol for the BRIGHT Intervention

6.1 Literature Review for Part Two of Thesis; Interventions Targeting Feeding Practices and Infant Weight Development in the First 1,000 Days

6.1.1 Why Intervene in the First 1,000 Days?

Childhood obesity prevention interventions and policy efforts largely fall outside of the first 1,000 days period.[162] The recent Cochrane review of childhood obesity interventions highlighted that less than 10% of randomised controlled trials (RCTs) in the field have targeted the early years of life (0-5).[162] Yet, a strong and burgeoning evidence base highlights the consistent relationship between RIWG and future overweight and obesity in later childhood and adolescence, outlined in Section 1.1.2 of Chapter One.[18,19] Moreover, supporting healthy patterns of weight development in infancy is of national priority as both weight faltering and RIWG have numerous implications for child health and development. Regarding RIWG, there is research showing RIWG is implicated in later cardiometabolic risk[29] and overweight and obesity[18,19]. Moreover, the prevalence of RIWG appears to be high in the UK population.[163] Whilst prevalence of RIWG is not measured in population-wide datasets, such as the National Child Measurement Programme, which only begins at age 4 - infant feeding trials and cohort studies have estimated that RIWG occurs in 18- 40% of infants.[24] Looking to population representative cohort studies – estimates have varied from 18% in the US[164] (n= 4626) to 46% from 0-6 months in Swedish cohorts (n=1780)[165]. Moreover, in a recent trial of infant feeding in the UK, ~40% of formula fed infants experienced RIWG (n= 669).[163] Whilst estimates of RIWG remain difficult to estimate at the population level, rates appear to be high, and therefore may be a promising potential target for the early emergence of overweight and obesity. However, at present there are no guidelines for the prevention, detection, or management of RIWG in the first two years of life in the UK.[26,166] Moreover, qualitative research with HCPs in the UK (n=116) demonstrated that a lack of shared understanding for dealing with early years' obesity, uncertainties in the recognition of 'risky' weight gain, and fears around harming the HCP and parents relationship, acted as barriers to discussions regarding early weight development.[167] Similarly, another study of 20 Primary Care Providers (PCPs) in the US reported the need for additional support and guidance to improve their ability to discuss and support excessive weight gain for infants under the age of two.[168] These reports are unsurprising given the limited resources and guidance regarding both the concept of RIWG and strategies to prevent RIWG provided to HCPs and early care providers in the UK. It has therefore been proposed that addressing RIWG and its related behaviours may be a promising target for the early emergence of overweight and obesity – yet few interventions have been delivered or evaluated in the early years period. There is an urgent need to explore (i) whether the risk of RIWG can be modified through intervention, given the limited number of interventions in infancy; and (ii) how such interventions should be delivered to ensure acceptability and feasibility to caregivers.

Target populations for feeding interventions in the first year of life

Evidence suggests that RIWG is particularly prevalent amongst formula fed infants. For instance, in the Baby Milk trial, which consisted exclusively of formula fed infants, 40% of the sample experienced RIWG in the first two years.[163] Given that formula feeding is highly prevalent in the UK (76% of infants in the IFS were exclusively formula fed or formula fed in combination with breastfeeding by 6 weeks of age[32]) these infants may be a particularly important group in whom to prevent RIWG. Moreover, recent evidence from the Baby Milk trial highlighted the widespread prevalence of overfeeding as majority of infants in the trial were offered volumes of milk above the WHO guidelines for formula feeding.[163] There are also numerous qualitative studies from mothers and caregivers reporting lack support and reputable guidance on formula feeding both in the UK[169–171] and other high-income countries such as Australia.[172]

In a recent systematic review of mothers' qualitative experiences of formula feeding (n=23), mothers reported the inadequacy of information and support for formula feeding as compared to breastfeeding, alongside emotions of guilt, concern, and a sense of 'failure' when ceasing to breastfeed exclusively exclusive breastfeeding.[171] These perceptions and emotions are rooted in a historical context: in the UK there is a strong public health focus on exclusive breastfeeding; at the same time, healthcare professionals worry that providing information to parents about bottle-feeding might undermine breastfeeding efforts, or will contravene the WHO International Code of Marketing of Breast-milk Substitutes.[167,168] For example, WHO's guidelines on providing parents about formula feeding state that formula feeding guidance should not be on general display or in leaflet racks in healthcare settings, and support should only be provided to caregivers once a 'firm' decision to formula feed has been made. [139] Moreover, in a recent review of the WHO's Baby Friendly Initiative to promote breastfeeding - which seeks to improve breastfeeding initiation, duration and support by offering breastfeeding interventions and restricting the use of breast milk substitutes - mothers felt that health professionals sometimes withheld formula feeding guidance to adhere to the initiative, leading them to feel unprepared and isolated when beginning to formula feed.[173] Moreover, mothers who took part in the Baby Milk trial felt that professionals were reluctant to provide them with support for formula feeding as to not deter breastfeeding efforts, and felt that formula feeding support need be easily available from the onset of formula feeding prior to the establishment of 'hard to change' feeding routines.[174] However, formula feeding – whether exclusively or in combination with breastfeeding – is introduced for a wide range of reasons, some of which may be unavoidable or outside of the control of a caregiver.[152] For example, formula milk may be introduced due to health-related complications of the mother or the child. However, given the limited resources and support for bottle-feeding currently available through the healthcare system and reputable sources, caregivers are left to seek guidance from less reputable sources from online platforms.[175,176] In a comprehensive review of online digital resources for infant feeding, the guidance and support available was often lacking evidence-based information and was even found to include information that contradicted infant feeding guidelines and therefore may pose potential risks for infant health.[177] The authors argued that evidence-based resources for formula feeding that are rooted in guidelines and best practice are needed to ensure caregivers are not left to rely on potentially unreliable guidance. Moreover, the limited guidance on formula feeding available to parents, and the little focus on formula feeding practices within these guidelines, may have important consequences. For example, in one Australian Study (n=153) nearly half (46.3%) of parents incorrectly prepared the formula (e.g. using tightly packed scoops or incorrect water to powder ratios) and only 18.9% received formula feeding guidance before using formula.[178]

Taken together, there is a critical need to support caregivers with evidence-based guidance on formula feeding – not only to address the lack of support presently available, but also to help support healthy growth amongst formula fed infants who appear to be at greater risk of RIWG.

Intervention options for feeding in the first year of life

One of the most important modifiable risk factors for RIWG is milk-feeding practices, as weight gain during infancy is closely related to energy intake. For instance, energy deposition as a percentage of total energy requirements are much higher at 1-month (40%) as compared to 12 months of age (1-2%).[179] Mechanisms that may explain the increased risk of RIWG amongst formula fed infants include both differences in formulation and the nutritional makeup of formula (the *what* of formula feeding)[109,180,] as well as the different feeding mechanisms and behaviours used when feeding an infant through a bottle versus from a breast (the *how* of formula feeding).[50] In a recent review of studies (trials and observational studies) exploring infant feeding practices associated with RIWG amongst formula fed infants, several of these 'how' mechanisms were identified.[144] For example, using 'follow-on' formula with a higher protein content (after the introduction of complementary feeding) as opposed to continuing to use 'first' infant formula with a lower protein content, using larger bottles, offering larger number of feeds, and adding cereal to bottles were identified as 'risky' formula feeding behaviours. Hence, the authors concluded that despite the limitations of the commonplace observational designs in this area, reducing the usage of risky formula feeding behaviours is important amongst infant feeding strategies and obesity prevention interventions. Moreover, formula-fed infants may consume a higher volume of milk than breastfed infants on average – with one large study (n=1,106) demonstrating that 71% of formula fed infants consumed more milk than their required intake on 3 or more of their first 7-feeds[181]. This suggests that feeding volumes of formula above the guidelines is pervasive in the early days of life. However, it's important to consider here that parents may be particularly motivated to help their infant regain their birthweight in this period and be learning how to observe their child's satiety cues, and therefore more likely to offer more milk or encourage the infant to continue feeding beyond satiety in this period. Whilst it is difficult to observe whether this overconsumption of milk is limited to bottle fed infants, as accurate estimations of milk intake from the breast is challenging, it has been postulated that overfeeding amongst formula fed infants may stem from the use of less responsive feeding practices when feeding through a bottle versus the breast.

Definitions of 'responsive feeding' vary across the literature, yet it can be described as a child-led approach to feeding where caregivers i) offer an age-appropriate volume of milk in response to their child's hunger cues and ii) cease feeding in response to their infant's satiety cues and iii) do not offer milk for purposes apart from hunger (e.g. feeding to soothe an unsettled but not hungry infant).[182] It follows that offering milk in absence of hunger cues, say to soothe an infant or encourage them to sleep for longer, or even pressuring a child to finish a bottle past their demonstrated satiety cues, would be considered 'unresponsive' feeding. It has been hypothesised that the persistent use of such unresponsive feeding practices may gradually disrupt an infant's ability to self-regulate their energy intake, according to their hunger and fullness, over time.[161] This can result in a cycle whereby a child is both offered an excess of milk and becomes less able to express fullness cues for their caregiver to cease feeding.

There are several observational studies demonstrating associations between unresponsive feeding practices and faster early infant weight gain [64,76] pointing to unresponsive feeding practices as targets for intervention. However, as discussed previously, such designs may mask reverse causation, where unresponsive feeding practices may be performed in response to early weight patterning, the temperament of the child, or their appetite. However, in recent years evidence from more rigorous experimental studies have demonstrated that responsive feeding interventions have resulted in more responsive feeding and more advantageous weight patterning. [183] These results will be discussed in the next section.

6.1.2 Previous Face to Face Interventions

In recent years, a few RCTs have been undertaken to explore whether supporting formula feeding caregivers with evidence-based guidance on appropriate and responsive infant feeding practices can improve weight and health outcomes for children. These trials have provided the first evidence that face-to-face support on responsive feeding, and related behaviours, may reduce the prevalence of RIWG in infants.

Baby Milk[163] was undertaken in the UK amongst healthy birthweight infants who received formula milk within the first 14 weeks of birth. Participants were randomised to intervention (N=340) or an attention matched control group (n=329). The intervention group received 1:1 sessions with a facilitator who provided guidance on formula milk intakes and responsive feeding, as well as growth monitoring to prevent rapid weight gain up to 6-months of age. In the evaluation of the trial, the intervention group reported reduced milk intake at 3 months of age (-14% as compared to control group) as well as at 4 (-12%), 5 (-9%), and 6 (-7%) months of age.[24] Moreover, the intervention group showed slower and steadier infant weight gain from baseline to 6-months as compared to the control group (mean change 0.32 vs 0.42 weight for age SDS), during the intervention period.[163] However, there was no significant difference in weight gain between groups from baseline to 12-months of age, suggesting the need for infant feeding interventions to be sustained past 6-months of age. However, the trial showed marked improvements in reported milk intakes in the intervention group as compared to the control group – crucial given the prevalence of feeding above the guidelines. As depicted in **Figure 6.1**, the intervention group more closely followed the pattern of recommended milk intake provision across the first 8 months of life, although they reported similar milk provision at baseline.[65] The researchers therefore proposed that that interventions designed to guide formula milk intake volumes, with feeding support, can effectively reduce overfeeding amongst formula fed infants.

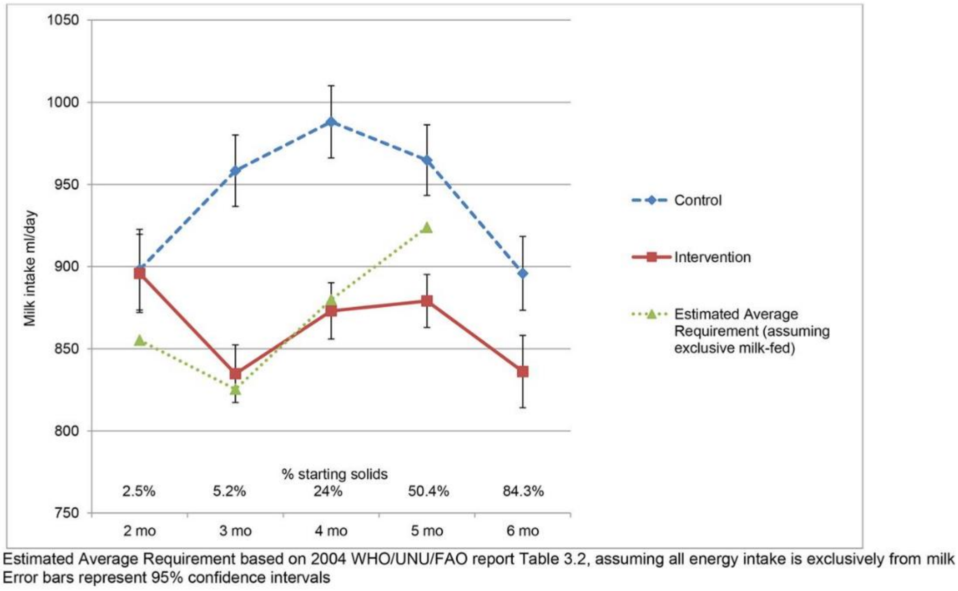
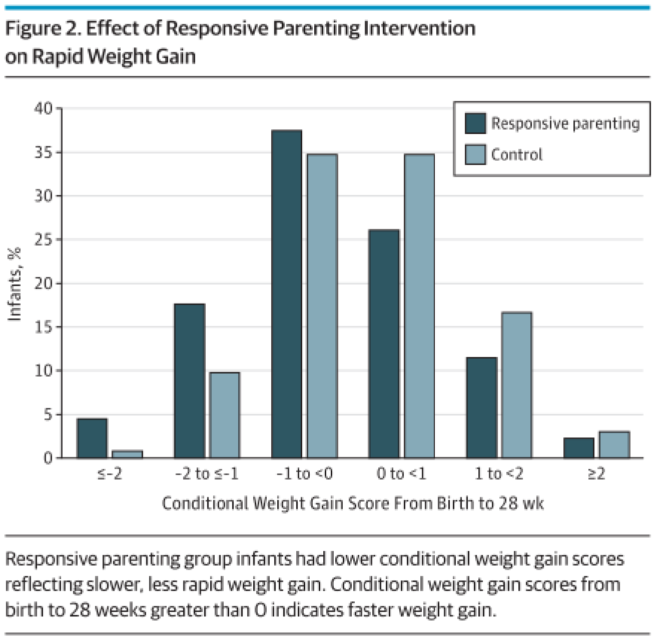


Figure 6.1. Reported milk intake (ml/day) in the intervention group as compared to the control group obtained from Lakshman et al[65]

Further follow up analyses of Baby Milk also showed that provision of the intervention improved maternal attitudes towards infant feeding guidance, which in turn promoted more responsive feeding behaviours.[184] Specifically, more positive attitude towards infant guidelines promoted by the intervention, was associated with caregivers offering smaller amounts of milk and slower infant weight gain. Crucially as well, these positive attitudes attenuated relationships between riskier infant appetitive traits, such as higher food responsiveness and lower satiety responsiveness, and infant weight. Hence, supporting parents to utilise infant feeding guidelines may not only prevent overfeeding across infants in general, but may be particularly beneficial for parents with infants expressing more avid and challenging appetite where overfeeding might be at higher risk. Taken together, the Baby Milk trial demonstrated that through face-to-face guidance, formula feeding practices can be modified, and that these led to some measured reduction of RIWG.

INSIGHT (Intervention Nurses Start Infants Growing on Healthy Trajectories) was a trial undertaken in the United States, in which mother-newborn dyads (n= 291) were randomised to an attention matched control and intervention group.[185] The intervention group received home visits at 3-, 16-, 28- and 40-weeks including face-to-face support with infant feeding, sleep, active social play and growth monitoring. INSIGHT included both breastfed and formula fed infants. The intervention set out to teach caregivers how to identify and respond appropriately to infant hunger and satiety cues, and prevent restrictive or pressured feeding practices when milk feeding from either the breast or from a bottle. Compared to the control group, the intervention group showed significantly slower weight gain from 0-7 months (0-28 weeks: −0.18 in weight SDS) and had a significantly lower weight for length percentile at 1 year of age (57.5% vs 64.4%).[183] Notably this effect did not differ across feeding modality, with both breastfed and formula fed infants in the intervention groups showing more favourable outcomes as compared to the control group.



Conditional Weight Gain Score from Birth to 28 Weeks; calculated standardized residuals from linear regression models of weight for age at 28 weeks (IV) on weight for age at birth (DV), including length for age at birth and 28 weeks and infant age at the 28-week assessment as covariates. This score therefore represents variation in child weight gain not explained by child age, birth length, or birth weight, a score of zero represents the population mean and positive scores indicate more rapid weight gain, while negative indicate slower weight gain.

Figure 6.2. Effect of INSIGHT Responsive Parenting Intervention on Occurrence of Rapid Infant Weight Gain obtained from Savage et al[183]

Of note, INSIGHT used a ‘stealth’ approach to reduce RIWG, by supporting responsive parenting behaviours across varying domains of infant care (e.g. sleep, temperament) and focusing more on ‘responsive parenting’ as opposed to overt messaging about rapid weight gain prevention. This approach differed from Baby Milk, which introduced RIWG and its obesity risk more overtly to parents. Given the effectiveness of both these trials in reducing rates of RIWG, it remains unclear whether a 'stealth' or overt approach to obesity prevention is more acceptable and engaging to parents. Nonetheless, both these trials provide convincing evidence that feeding guidance and interventions can lead to promising outcomes for infant weight development.

The NOURISH trial in Australia (n=698) promoted[56] responsive feeding later in infancy, namely during complementary feeding. Mothers were randomly allocated to receive either usual care or attend group education modules focused on responsive feeding at both 4-6 and 13-15 months of age, each over the course of 3-months. Whilst this intervention focused largely on responsive feeding in relation to complementary feeding after ~6-months of age, it also provides experimental evidence that feeding practices can be modified through face-to-face intervention, which in turn may influence weight outcomes. For instance, the control group showed a significantly higher risk of RIWG between birth and 14 months of age (OR=1.5, 95% CI=1.1, 2.1). In addition, parents in the control group were significantly more likely to use unresponsive feeding practices such as using food as a reward (15% vs 4%).[140] Moreover, at 3.5 years of age, mothers in the intervention group continued to use more responsive feeding practices on 6/9 feeding subscales.[186] suggesting that the early provision of responsive feeding guidance can lead to sustained improvements in feeding behaviours over several years.

In recent years, more face-to-face trials have sought to develop responsive feeding interventions targeted at high-risk populations such as those with more limited financial and social resources. In the US, the Sleep SAAF intervention (n=194)[187] adapted the INSIGHT trial to be tailored to African American mother-infant dyads, as these populations report more un-responsive feeding practices and are more likely to be fed with formula milk[188], and face a greater risk of RIWG[189]. The intervention group received a nurse-delivered face-to-face curriculum that provided guidance on sleep, feeding, soothing, and active play which was tailored towards the preferences and barriers to responsive feeding amongst African American families. Overall, infants who received the intervention were nearly half as likely to experience RIWG (14.1% vs 24.2%; OR = 0.52, 95% CI: 0.24, 1.12), as defined as an upward centile crossing of two weight-for-age percentile lines (>1.34 z-score increase) compared to control infants.[190] Moreover, intervention mothers reported more responsive bottle-feeding practices, such as using less pressure to finish the bottle, use of cereals to soothe, and using other beverages to soothe. These findings support the overall utility of formula feeding interventions, and suggest that targeting interventions towards higher risk populations may be effective at improving health equity in early health and development.

However, to date, smaller trials with less comprehensive interventions of a shorter duration have shown less effective. The Mothers & Others (n=430) trial, also in the United States, did not lead to significant differences in infant growth or rates of RIWG at 15-months of age, following a home-based responsive feeding curriculum tailored to non-Hispanic African American families.[191] These findings mirrored a previous trial targeting low-income women using the Special Supplemental Nutrition Program for Women, Infants, and Children (WIC) in the US in which a single face-to-face session on responsive bottle feeding for mothers did not lead to significant improvements in infant weight development (n=38).[192] Finally, Reifsnider et al’s trial (n=119) targeted pregnant Latina women with obesity using the WIC programme, and offered additional home visits by community workers focused on infant growth, breastfeeding nutrition, sleep and physical activity.[193] The trial did not show significant differences in weight-for-length z-scores or overweight classification at 12-months of age. One explanation for these null findings could be that the control groups access to WICs peer counsellor programme may have limited the interventions' ability to show marked differences in weight outcomes. Moreover, interventions focusing on breastfeeding support, such as Reifsnider, without adequate support for formula feeding parents, may result in less marked improvements in infant growth outcomes. Together, a few trials to date have produced varying conclusions as to whether face-to-face interventions targeting high-risk and more deprived populations are effective at improving infant growth outcomes and therefore future obesity risk. Whilst it is difficult to extrapolate given the varied populations, intervention materials and timelines of these interventions, it appears that more comprehensive interventions which offer support over an extended period may be more effective in promoting behaviour change and improving infant growth outcomes. it is of note that to date, only one infant feeding trial – Baby Milk – has been conducted in the UK.

6.1.3 A Case for Digital Interventions

The trials targeting infant feeding practices presented so far have all been delivered face-to-face, which may hinder scalability and implementation across the population. Face-to-face interventions are expensive and place additional burdens on national healthcare systems, which in the UK is increasingly understaffed. Especially in the wake of the Covid-19 pandemic, providing more cost-effective, easily accessible, and scalable interventions may be a promising approach to promote health equity in infant feeding practices and growth outcomes. Moreover, it is becoming increasingly commonplace for caregivers to seek guidance through online platforms and apps, such as websites, Instagram, and YouTube.[176,177] This is particularly relevant to formula feeding parents, who are left to seek information from such sources given the lack of formula feeding guidance available through the National Healthcare system or other professional organisations.[171,175] Moreover, parents often express a desire for reputable online sources of support. For instance, in the Norwegian Early Food for Future Health (n=718), 80% of parents who participated in an intervention with web-based videos, reported viewing all/most of the video clips on infant feeding. [194] Moreover, parents preferred using the internet to guide them on infant nutrition but felt that public authorities should be involved in the creation of such resources. In addition, a recent study with parents of Australian children aged 2-5, reported that online platforms were their preferred method for participating in feeding interventions.[195] Moreover, parents were willing to engage in longer interventions when delivered digitally, for around 12-weeks, as compared with only 4-weeks for a face-to-face intervention. Whilst we cannot directly extrapolate these perspectives to parents of infants, it is plausible that these views may be shared by many parents given the demands of face-to-face interventions on time-poor parents of infants, who may face difficulties attending sessions outside of the home with a very young infant. Qualitative work with parents on digital information seeking has demonstrated parental concern about the reliability of health information, particularly amongst commercial product websites.[175,176] This is particularly relevant to infant feeding, as reputable sources of guidance are scarce in the UK, yet freely available online sources of information, such as blogs, apps, and YouTube videos, and content provided by formula milk companies are ubiquitous. In a recent Australian evaluation of 47 publicly available apps for infant feeding, which were not endorsed by a healthcare or government body, 53% were deemed to be incomplete or provide incorrect information, and 64% included information which did not align with the Australian government guidelines.[176] Given how abundant and easily accessible such sources of information are to parents, it is crucial to provide access to reputable and evidence-based guidance to counter the mass of unreliable information.

In the UK, the NHS provides detailed and comprehensive digital resources on infant care. Through NHS.co.uk and other endorsed sources, parents can access information on immunization, safe infant sleeping, breastfeeding and many other topics. However, resources and guidance on formula feeding or bottle-feeding is notably scarce, particularly in relation to the behavioural aspects or the 'how' of formula feeding.[196] Moreover, there have been a plethora of digital interventions seeking to promote health behaviours and change feeding behaviours in later childhood.[197] In a recent review of web-based interventions designed to change parental feeding practices, 11 studies were undertaken, mostly (n=8/11) in the United States.[198] Whilst the review included parents of 0–12-year-old children, six interventions targeted parents of 2-6 with the rest over 6 years of age. Overall, the review highlighted small programme effects across the varied interventions. Moreover, in evaluation of the Behaviour Change Techniques across the COM-B Models often used in behavioural interventions, interventions most often targeted the capabilities of parents, using instruction on how to perform and demonstration a behaviour.[199] Whereas BCTS targeting motivation such as goal setting and feedback and monitoring were used less often, despite self-regulatory strategies playing a critical role in enacting behaviour change.[199] The authors also highlighted the lack of individualized feedback across the interventions – likely due to its higher demands on parents in the form of data collection. Whilst technology allows for tailored and immediate feedback according to parental responses – which may provide guidance that is particularly relevant to that parent – it demands that enough information be provided to allow for reliable tailoring. Although more tailored approaches in interventions are promising, it can be difficult to strike a balance between increasing participant burden to collect sufficient information for tailoring.

Together, this review highlights the lack of interventions targeting responsive formula feeding as well as the need to integrate self-regulatory strategies and tailored elements into such interventions.[200] Developing and providing evidence-based digital guidance on responsive formula feeding practices may therefore be a practical, cost-effective, engaging and preferred way to support parents with formula feeding best practices. In a similar vein, a digital intervention may support harder-to-reach populations living in greater deprivation, as they are more easily accessible than face-to-face interventions, and freely available. To date, no digital interventions have been developed or delivered to support formula feeding practices, specifically, and related behaviours in infancy in the UK.

6.1.4 Barriers and Facilitators to Responsive and Appropriate Formula Feeding Behaviours

Promoting responsive feeding behaviours may be a critical component of successful childhood obesity preventions, as described above. For instance, supporting caregivers to respond to their child's hunger and satiety cues may help to promote infants' self-regulation of energy intake and observational studies demonstrate that infants of parents who perform more responsive feedings demonstrate more favourable weight trajectories. Moreover, interventions aimed at improving child health and which have targeted responsive feeding have shown larger improvements in child weight and feeding behaviours than those without a focus on responsive feeding.[201] Nonetheless, few studies have been undertaken to explore *how* to successfully target and change responsive feeding behaviours in infancy.

As highlighted by the COM-B Model[202], behaviour changes when an individual's capability, opportunity, and motivation to engage in a specific behaviour are targeted. Moreover, theoretical models such as COM-B Model highlight the need to explore barriers and facilitators to components to enact behaviour change. One review to date[182] has sought to systematically explore barriers and facilitators to responsive infant feeding, utilising the COM-B Model. A total of 36 studies were reviewed, which were largely qualitative in design and undertaken in the US (n=20) and the UK (n=8). Barriers and facilitators to formula feeding responsiveness will be summarised below.

Regarding psychological capability (C of COM-B), four enablers were identified: i) responsive feeding skills; ii) knowledge and understanding of feeding, appetite, and nutrition; iii) caregiver attitude to who controls feeding; and iv) education to support responsive feeding. This suggests that supporting caregivers to recognise their infant's unique hunger and satiety cues is crucial to changing responsive feeding behaviour. Secondly, in six of the eight qualitative studies, understanding infant appetite was often reported as an important component of responsive feeding, which might suggest that tailoring responsive feeding advice to infant appetite, is important and helpful for parents. Finally, some studies identified a more controlling or rigid attitude to feeding, where parents might choose to override hunger and satiety cues, was a barrier to responsive feeding and must be addressed through intervention.

Several aspects of physical opportunity (O of COM-B) were also identified as important: i) the influence of the physical environment on parental responsiveness; ii) mother–infant physical contact; iii) maternal distraction to physical objects during feeding; and iv) structural/environmental factors. Firstly, elements of the broader physical environment were discussed as a barriers to responsive feeding, with examples being visible measurements of bottles of formula milk and instructions on formula milk packages. Whilst these prompts to guide parents to offer age-appropriate volumes of formula cannot be removed from the environment, supporting parents to interpret this guidance in the context of their child's appetite and hunger cues, could help support responsive feeding practices. This could involve presenting guidelines as an approximate guide, and highlighting that these suggested amounts are not goals to aim for, and should not override their infant's expressed satiety cues during feeds (e.g. pressuring an infant to finish the bottle). Similarly, some studies highlighted structural environmental factors, such as low income, as barriers to responsive feeding if parents are concerned about food wastage or future scarcity (e.g. concern about wasting formula milk left in the bottle that cannot be used again). Whilst these distal environmental factors cannot be directly targeted through digital health interventions, ensuring that families living under more deprivation have access to, and are consulted in, the development of such interventions is important. Five social opportunities (O of COM-B) were also identified: (1) advice and support; (2) social and cultural norms and expectations; (3) child cues; (4) influence of the social environment on caregiver response; and (5) interactions with child during feeding. Critically, parents felt the lack of support offered by healthcare professions was a key barrier to responsive formula feeding. Similarly, the cultural norm or perceived expectation to breastfed over formula feeding was also a barrier as parents expressed significant stigma, even from healthcare professionals they sought support from. Whilst these socially engrained stigmas are difficult to address, supporting parents with neutral and non-stigmatising guidance, they might not otherwise be able to access easily, may be key to overcoming stigma and promoting responsive formula feeding.

Four barriers and facilitators were considered important aspects of reflective and automatic motivation (M of COM-B: i) beliefs about consequences of parental feeding practices; ii) feeding goals, intentions, and plans; iii) caregiver emotions; and iv) parental internal cues. Whilst goal setting and self-monitoring are established as useful for dietary behaviour change in adult populations, it remains unclear whether such BCTs may promote responsive feeding or inadvertently promote more controlling feeding. For instance, encouraging parents to set marked feeding goals might encourage them to override infants' hunger and satiety cues in favour of these goals. Hence, the influence of these BCTs in infant feeding must be explored and considered carefully in future interventions. Secondly, parental emotions such as distress was identified as a key barrier, suggesting that supporting parental wellbeing and mental health outside of feeding guidance may be an important element of interventions. It also underlines the importance of formula feeding guidance being non-stigmatising and supportive in tone., A recent review of the UK's implementation of the WHO's Baby Friendly Hospital Initiative[173] found short-term improvements in breastfeeding duration alongside negative emotional experiences. Five qualitative studies in this review highlighted how caregivers/mothers often felt criticized and judged when integrating bottle-feeding or formula feeding, often leaving them with reduced feelings of self-efficacy. Moreover, the commonplace pro-breastfeeding discourse led to feelings of guilt, inadequacy and self-blame, and even cases of post-natal depression, in cases where mothers could not breastfeed or chose not to. Hence, considering and buffering such negative emotions that result from a largely pro-breastfeeding culture should be central to forthcoming interventions targeting formula feeding families.

Finally, some barriers to responsive feeding emerged outside the COM-B Model. For instance, child weight, with both high and low child weight, were reported alongside more pressuring and restrictive feeding styles respectively. This finding might reflect important reciprocity in child feeding, such that caregivers modify their feeding in line with their child's growth – pressuring a child who is growing more slowly or showing weight faltering and restricting a child who is gaining weight rapidly. Hence, feeding an infant 'responsively' in the face of concern for appropriate growth may be a significant challenge. Therefore, supporting parents to refrain from using unresponsive practices, which could in turn establish unresponsive patterns in future, in the context of weight concern should be explored in future interventions. Take together, the presented review pinpoints seven key barriers and facilitators, which may be targeted and explored further in the development of novel infant feeding interventions.

6.1.5 Tailored Infant Feeding Guidance: A promising avenue for interventions?

Tailored guidance holds the potential to enhance the quality of infant feeding support and improve outcomes for infants and caregivers alike. In addition to weight patterning[40,70], PFPs have shown to be responsive to infant characteristics and appetite traits in few observational stuides.[73] Moreover, follow up analyses of NOURISH found that mothers whose infant had a more challenging temperament at 7-months of age, reported a lower awareness of infant hunger cues, higher frequency of using food to soothe, and a higher concern for overweight and underweight [203] Hence, caregivers of infants who are more difficult to soothe when crying, and who are less adaptable to new exposures and activities, may benefit from guidance that is tailored to their needs and experiences. Moreover, in Gemini (n=1950), parents whose 3-month-old infant had a smaller appetite used more pressuring PFPs, while those whose infant had a larger appetite were more restrictive. [73] Similarly, in later childhood, parents have been shown to use more controlling and restrictive PFPs with children they perceive to have a more avid appetite, to manage their child's food intake.[204] On the other hand, parents of fussy eaters – characterised by an unwillingness to try new foods or strong food preferences – use more pressuring PFPs in order to encourage their child to eat a larger amount or greater variety of food.[205] However, once again these findings come from largely observational cohorts, in which it is difficult to disentangle the individual determinants (i.e. appetite traits) from the shared environmental determinants (i.e. socio-economic status) of PFPs. The discordant twin design offers a powerful design to identify individual child traits that might influence parental feeding decisions and be targets for intervention.[35] Using this approach in Gemini, I co-authored a paper demonstrating that parents used more pressure for a co-twin demonstrating lower food responsiveness, food enjoyment, emotional overeating as well as higher satiety responsiveness, slower speed of eating and greater fussiness in toddlerhood.[82] This study is one example of an emerging evidence base, presented above in section 1.2.3 of Chapter One, demonstrating how parents modify their feeding practices based on their child's specific appetite and weight development. Therefore, it has been proposed that offering *tailored* feeding guidance that considers the child-driven challenges that parents might face when attempting to feed responsively might be promising. Whilst few studies have explored the acceptability and feasibility of such approaches with caregivers, previous qualitative insights from caregivers highlights how a 'one-size fits all' approach to feeding guidance and support can be frustrating and less welcomed than a more tailored approach.[173] Hence, future studies are needed to explore caregiver perceptions of tailored infant feeding guidance, as well as potential implications of such interventions for infant feeding and perhaps even infant weight development.

6.2 Aims

6.2.1 Introduction to the BRIGHT Study

BRIGHT is a novel digital intervention which aims to support responsive feeding and healthy infant growth amongst caregivers using formula milk to feed their infants. At present, there is a lack of comprehensive, non-commercial, and reputable guidance for formula feeding available for families in the UK. As a result, parents are left to less reputable sources of guidance, such as information provided by formula milk companies or social media, the latter of which has been shown to provide unreliable and sometimes incorrect information[177]. Moreover, qualitative reports highlight how the lack of formula feeding support available to families often leads to negative emotions – such as guilt, stigma, and reduced self-efficacy.[171,173] Therefore, there is a pressing need to develop and deliver evidence-based formula feeding guidance that is free from commercial influence. Nonetheless few existing face-to-face interventions, such as Baby Milk, NOURISH and INSIGHT have demonstrated the potential to intervene upon bottle-feeding behaviours to promote more favourable weight development across infancy.[140,163,183] However, face-to-face interventions are costly and difficult to scale across the population. A digital intervention would have the advantage of being scalable to the population level, cost-effective and sustainable over the longer-term. Therefore, BRIGHT seeks to adapt the Baby Milk Trial and NOURISH trials into a digital format, to be delivered through the Baby Buddy app. BRIGHT specifically seeks to encompass both the *'what'* of formula feeding (e.g. feeding amounts and choosing the right type of formula) as well as the '*how'* of formula feeding (e.g. recognising hunger and satiety cues and responsive bottle feeding) with a focus on responsive bottle-feeding. Although complementary feeding behaviours (recommended to start at about 6-months of age)[26] will be included in the wider BRIGHT protocol, I present the milk-feeding modules of BRIGHT (formula feeding, sleep, crying and growth) given my PhD's focus on milk-feeding in infancy.

6.2.1.1 The BRIGHT team

The development of BRIGHT was led by academic experts across UCL (Dr Clare Llewellyn, PI; Prof Atul Singhal (to July 2021), the University of Cambridge (Professor Ken Ong, Co-I; Dr Raj Lakshman, Co-I; Dr Andrea Smith, Co-I; and Dr Amy Ahern, collaborator) as well as collaborations from the University of Southampton (Dr Kathryn Bradbury) and Queensland University of Technology (Professor Lynne Daniels and Dr Rebecca Byrne). Alongside the team of investigators, several researchers and students have been involved in varying stages of BRIGHT's development, including MSc Health Psychology students (Celina Cimino, Tiffany Denning, Zohar Preminger, Sylvie Majorova and Anna Sochiera), PhD students (myself and Dr Alex Rhodes), a Research Assistant (Sarah Esser) and an intern (Isabel Richie). Where external expertise could be utilised to enhance BRIGHTs development, additional expertise was sought from health care professionals, policy makers, and external academics. The BRIGHT project is funded by a UK Medical Research Council PHIND Grant (MR/T002700/1).

6.2.1.2 Best Beginnings and Baby Buddy

The BRIGHT intervention was developed to be hosted in the Baby Buddy 2.0 app, leveraging its existing user base and network of young families and healthcare professionals. Baby Buddy is a freely available parenting app that supports caregivers from pregnancy and throughout their baby's first weeks of life. Baby Buddy was developed and is overseen by the charity Best Beginnings, a key partner in the development of BRIGHT. The original version of Baby Buddy was developed by UK charity Best Beginnings with funding from the Big Lottery and was launched in 2014. Baby Buddy 2.0, funded by the National Lottery Community Fund and the Fidelity Foundation was launched in 2021. Baby Buddy 2.0 was re-launched to include guidance up to a child's first birthday. Baby Buddy 2.0 (hereafter referred to as Baby Buddy) consists of written and video-based resources (over three hundred films) to support caregivers with feeding, infant care, monitoring infant growth, as well as supporting their own mental health and wellbeing. Moreover, the app provides caregivers with a daily bite-size and personalised message containing guidance relevant to their stage of gestation or baby's age. Critically, Baby Buddy is integrated into the maternity care pathways of around 50 NHS Trusts across the UK and endorsed by eight Royal Colleges and Professional Bodies. Moreover, Baby Buddy utilises an editorial board with representatives from endorsing organisations and experts in child health across the UK, which review new content before it is uploaded onto the app to ensure it is evidence based. In a recent review of 29 UK based pregnancy apps, Baby Buddy was one of only two apps that contained no inaccurate information.[206] However, Baby Buddy does not contain comprehensive guidance regarding feeding infants with formula milk, nor guidance regarding responsive feeding in the context of bottle-feeding.

Baby Buddy is an ideal platform for BRIGHT for several reasons. Firstly, it is a free, non-commercial (advert-free), and multi-award-winning platform, which is integrated into and used widely within the National Healthcare Service in the UK. It can be downloaded freely from the NHS library, and materials are produced to be of a reading age of 12 years to maximise the accessibility of written resources. Crucially, Baby Buddy has a wide reach across the UK as it has been used by >350,000 parents, with an average of ~7,000 new users every month.[207] Baby Buddy has a significant representation of younger mothers, black and ethnic minority (BAME) parents, and families from a lower SES background[207]. As previous reviews of responsive feeding behaviours have pointed to deprivation and socioeconomic contexts and barriers to the adoption of these behaviours[182], the representative population of Baby Buddy is crucial to BRIGHTs implementation. In line with this, Best Beginnings also takes a proportionate universalism approach to development, seeking to reduce inequalities by targeting those living in greater economic and social deprivation.[208] BRIGHT seeks to align itself with this approach. Baby Buddy also places significant emphasis on embedding itself into local communities by leveraging local leaders as Baby Buddy Champions, in order to tailor its delivery to the local healthcare system and increase its uptake. Moreover, Best Beginnings has an established network of stakeholders and healthcare professionals, who make up their editorial board. These stakeholders and experts can be leveraged in the development of BRIGHT to provide expertise as well as ensure that it is a feasible tool to be used within UK healthcare settings. Throughout the development process we worked closely with Best Beginnings' digital, content and engagement teams to translate academic insights and research into an engaging digital tool to support responsive feeding amongst caregivers.

6.2.2 Aims

For the final chapter of my PhD, I led on developing a protocol for the formula feeding resources within the Baby Responsive Intervention for Growth & Health Tracking (BRIGHT) , as funded by a Medical Research Council Public Health Intervention Development (MRC PHIND) grant and in collaboration with the UK Charity Best Beginnings. The current chapter will therefore detail the development of the digital BRIGHT resources related to formula feeding – including four Baby Buddy 'modules' on formula feeding, growth monitoring, sleep, and crying given the current thesis' focus on milk-feeding within infancy. In Study 1 of this thesis, I found that bottle-feeding, and it's associated mechanisms, might in part explain why infants fed with formula milk demonstrate a greater risk of experiencing RIWG. In Study 2, I also demonstrated important reciprocity between infant feeding practices and early infant weight gain. This suggests that interventions seeking to change feeding behaviours or support healthy growth should consider how parents might adapt their feeding practices in response to their infant's weight development in the first year of life. These insights were taken forward to develop the BRIGHT prototype.

The BRIGHT prototype has been developed in line with the PBA to digital health behaviour change intervention development[209], to ensure an intervention prototype that is acceptable, feasible, practical, and engaging for caregivers. We therefore aimed to undertake the first two stages of the PBA; i) planning and co-design and ii) optimisation to develop the prototype for BRIGHT. The third stage of the PBA, iii) implementation and evaluation, will be undertaken in a later phase of research. Under the first stage of the PBA, our planning phase aimed to; establish a partnership with Baby Buddy, acquire and identify gaps in resources from the Baby Milk Study, and perform a rapid scoping review of qualitative research to identify the barriers and facilitators to caregivers following formula feeding guidelines. We then undertook a second co-development phase to co-create the milk-feeding BRIGHT modules with a representative panel of PPIE caregivers. Finally, in our optimisation phase we sought to optimise a prototype of the milk-feeding BRIGHT modules using qualitative process analysis (or 'in the wild' study) as guided by the PBA.

6.2.2.1 My Unique Contributions to the BRIGHT prototype development

Throughout my PhD I led on the development of the milk-feeding resources of BRIGHT as specified below. Under the first stage of development, I undertook the behavioural coding of Baby Milk, drafted four of the six BRIGHT modules (formula feeding, sleep, growth, and crying) and drafted the video scripts for the formula feeding and growth modules. Moreover, I supervised the scoping review – led by intern Isabel Richie. Under the PPIE stage of research, I led on the recruitment and data collection using BRIGHTs PPIE Panel and implemented the prototype modifications in line with the PBA. I also drafted the prototypes for two of three interactive features; the formula feeding tracker and the tailored weight feedback, as well as led on the development of the Theory of Change model. In the final stage, I supervised the development of BRIGHTs prototype and the 'In the wild' qualitative study – both led by MSc student Zohar Preminger. Finally, throughout BRIGHTs development I have led on partnership and project management for the project – administrating the collaboration between the BRIGHT research team, Best Beginnings, as well as relevant stakeholders.

6.3 Methods

6.3.1 Overview of Development Process

The development of the BRIGHT prototype involved three phases of development in line with the first two stages of the PBA[209] – I) Planning and ii) Optimisation. The final stage of the PBA (implementation and evaluation) was not undertaken, as the current project set out to development the BRIGHT prototype for an evaluation trial in a future stage of research which would address this later stage. For the first stage of the PBA, we undertook two phases of research. Phase 1 (planning) aimed to establish the scope of the BRIGHT intervention by reviewing resources from the Baby Milk study, synthesizing existing literature on barriers and facilitators to following formula guidelines and responsive infant feeding, and agreeing with Best Beginnings how best to address current gaps in Baby Buddy content. Phase 2 (co-development) then aimed to engage a PPIE panel of caregivers and use its input to help co-create the new BRIGHT modules for Baby Buddy. For the second stage of the PBA, we undertook one phase of research. Phase 3 (optimisation) aimed to use explore user responses to the new BRIGHT modules in a prototype format, determining their appeal and relevance and identifying areas for improvement or amendment.

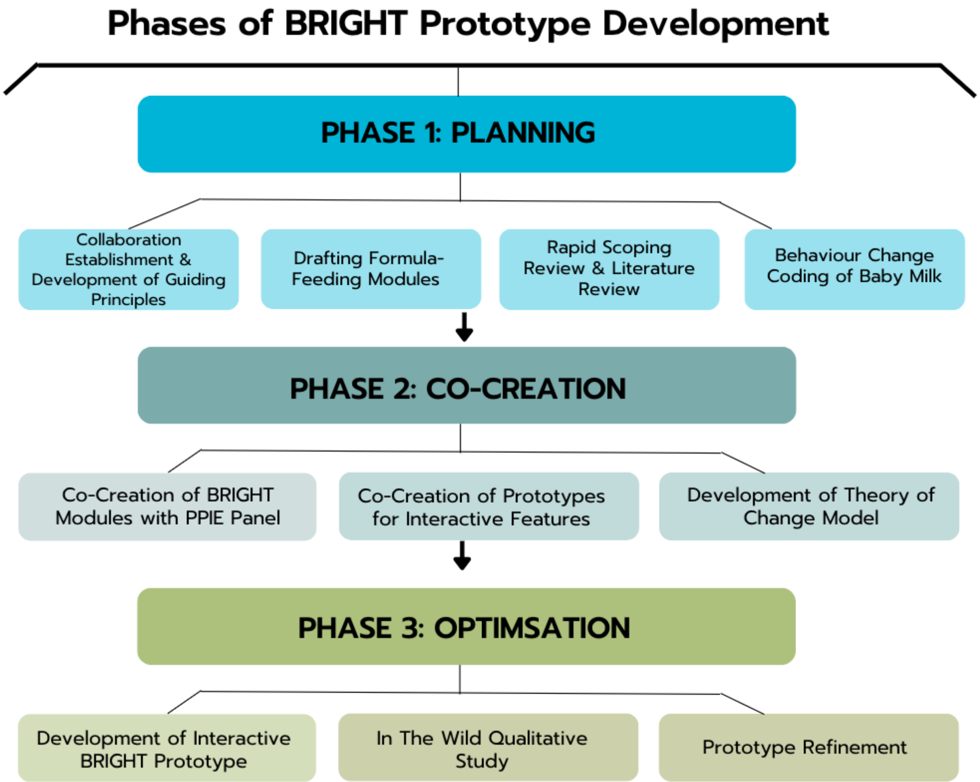


Figure 6.3. Flowchart depicting BRIGHT protocol development in line with Person Based Approach for Intervention Development

6.3.1.1 Target Population for BRIGHT

In the early phases of development, it was agreed with Best Beginnings that the BRIGHT prototype, and future trial, would be aimed at caregivers who are exclusively formula feeding their infants. This decision was made to ensure that breastfeeding efforts are not undermined by giving caregivers access to detailed information about formula feeding, in the critical early weeks during which breastfeeding is established. However, during the development of BRIGHT it became increasingly apparent that many parents using formula to feed their children in combination with breastmilk would not be able to benefit from these resources. Moreover, the Best Beginnings ‘parent panel’ of caregivers who inform the ongoing development of resources for Baby Buddy – increasingly raised the need for formula feeding caregivers to be better supported within the app, whether they are exclusively or partially formula feeding. Hence, we are currently in ongoing conversations with Baby Buddy to establish whether both mixed and exclusively formula feeding caregivers will be the target audience for the future evaluation of BRIGHT.

6.3.2 Approach and Theoretical Frameworks

The development of the BRIGHT protocol consisted of three stages, each rooted within the PBA[209]. In addition, to ground the development of BRIGHT in a theoretical understanding of behaviours targeted, we adopted the Com-B model and applied the Behaviour Change Wheel (BCW)[202], throughout intervention development. Finally, to evaluate and optimise the BRIGHT prototype in the final stage of research, we adopted the APEASE framework.[210] Each of these theoretical frameworks will be described in the following sections.

6.3.2.1 The Person Based Approach for Intervention Development

The PBA is a framework that grounds the development of digital health interventions in the experiences and psychosocial contexts of the populations who will use them. [209] It was initially developed to help address the low uptake and adherence rates commonplace across digital behaviour change interventions.[211–213] The PBA offers researchers a systematic, yet flexible process to: i) gain insight into the needs and experiences of users which an intervention seeks to support; ii) identify key characteristics and features that make an intervention more engaging, useful, and acceptable; and iii) iteratively design and refine an intervention using co-creation with target users. The PBA presents key stages that can be followed to achieve these goals, which can be adapted based on the needs of the intervention developers. Roughly, these three stages include; i) intervention planning ii) intervention optimisation and iii) implementation and evaluation. Moreover, central to the PBA is the development of a set of guiding principles, which seek to outline how the intervention will address key issues that may promote of hinder to engagement to the intervention. The guiding principles help ensure the researchers remain focused on the key goals of fast-moving and comprehensive interventions, and they should be revisited and refined throughout development.

Whilst each stage of the PBA consists of potential exercises outlined in **Figure 6.3**, these stages and exercises are not discrete and can be undertaken iteratively or within later stages of intervention development. The common steps and stages undertaken as part of the PBA are illustrated in Figure 6.4 Nor are these exercises strictly necessary where an intervention is adapting an existing intervention or qualitative insights regarding the target behaviours are already available. Hence, the PBA can accommodate for the presence, or lack, of quantitative or qualitative evidence for existing intervention components. For example, BRIGHT will largely be developed from existing formula feeding materials developed for the Baby Milk trial, which were created using in depth qualitative insights from caregivers.[174] Hence, we did not need to undertake in depth qualitative research to describe the experiences of BRIGHT's target users as they mirrored Baby Milk's. Instead, we chose to adopt participatory research methods to allow our target users to help us adapt the Baby Milk resources alongside the research team.[214] In this way, we could integrate target users lived experiences and perspectives into every piece of content adapted or development. Participatory research is generally defined as *collaborative* research performed with individuals who are the subject of study, allowing the target population to be active partners research processes and outcomes.[214,215] These methods have been as particularly important in the development of interventions affecting families and children, as they can increase attention given to marginalised and underserved communities and allow for these communities to gain agency in the policies and interventions which influence their day to day lives.[216,217] Given, the relative lack of support and resource available to formula feeding families, and the sensitivity and stigmas that surround formula feeding, we deemed participatory approaches critical to the development of BRIGHT. Moreover, the PBA also draws on qualitative methods that fall under the umbrella of "usability testing".[218] These methods utilise qualitative methodology to ensure a product is fit for purpose and adequately engaging by actively 'testing' the adoption and usability of an intervention in practice as opposed to in theory. This can help to counter the notoriously high drop of rates and low real-world engagement observed with digital health interventions.[212] Hence, we included an 'in the wild' qualitative study, for the optimisation phase. In this study, we asked our target-population to use a functional prototype of the BRIGHT milk-feeding resources over a two-weeks period in order to evaluate the resources in a real-world context, and over a longer period of time. Moreover, as the PBA not only seeks to ensure that digital interventions are fit for purpose, but also that they have potential to change target behaviours. This is achieved by considering and meeting the needs of users in the context in which they live and operate using behaviour change frameworks such as the Behaviour Change Wheel (BCW). This model was therefore adopted under each phase to ground BRIGHT in a theoretical understanding of appropriate and responsive feeding behaviours across infancy.

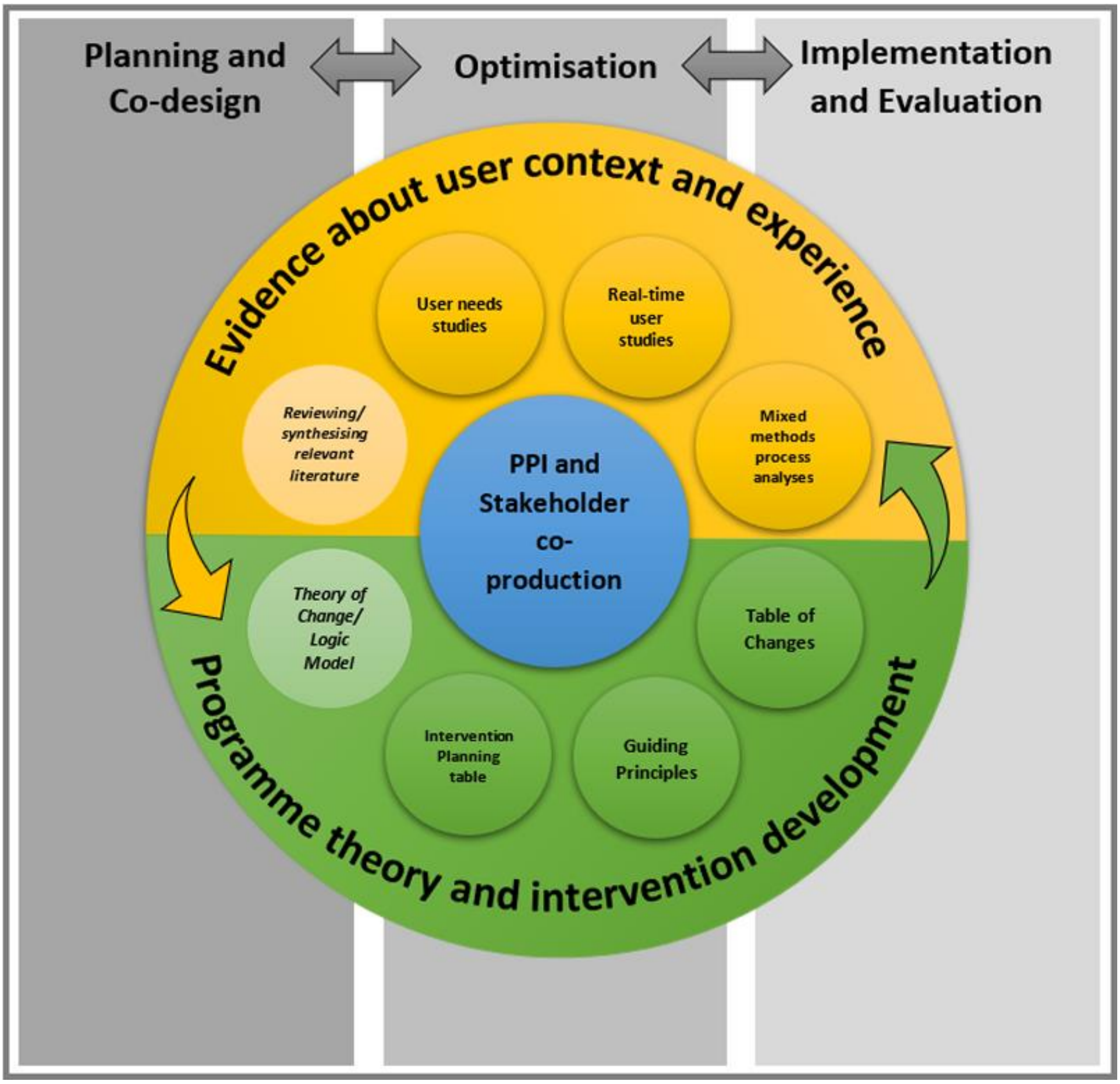


Figure 6.4. Diagram Illustrating the Stages of the Person Based Approach (PBA) for Intervention Development obtained from Yardley et al[209]

Stage One of the PBA: Planning and Co-Design

The first stage of the PBA – *planning* – seeks to highlight key behavioural targets in the context of the participants needs and challenges. This stage lays the groundwork for the intervention design and for relevant theoretical frameworks to be applied to the intervention. The BRIGHT intervention sought to adapt the previously developed Baby Milk formula feeding resources to develop the Baby Buddy formula feeding modules. As the Baby Milk resources had been developed using extensive qualitative research, co-creation, and feedback from formula feeding caregivers[174] , we did not undertake in-depth qualitative user needs studies prior to the co-creation process. However, central to the current planning stage was the identification of relevant theoretical models and behavioural targets from both previous interventions and the existing literature base. To achieve this goal, we undertook: (1) a rapid scoping review of literature exploring barriers and facilitators to adhering to the WHO guidelines on Formula Milk; (2) literature review of studies barriers and facilitators to responsive milk-feeding behaviour; and (3) a mapping exercise of Baby Milk using the Behaviour Change Technique (BCTs) from the Behaviour Change Wheel (BCW). Moreover, in this early stage we began the process of co-creating guiding principles – with relevant partners and stakeholders - which seek to operationalise the key intervention objectives of the intervention. As digital interventions can be complex and fast-moving, a set of key principles can help ensure the stages of development align with the overarching goals of the project.

Within the first stage of the PBA researchers can also begin to build their key intervention design components and features through co-design. Therefore we set out to 'co-create' the BRIGHT resources as a second step or 'phase' of the planning stage. This largely consisted of co-creation of intervention materials, to help improve the acceptability and feasibility of the resources from the users' perspectives. During co-creation, participants are asked to anticipate the needs of others as opposed to reporting on their own experiences and needs in relation to the intervention. Hence, we focused the optimisation phase on co-creation work using a PPIE panel of parents that represent the user population of formula feeding caregivers. Moreover, in line with the PBA, the planning may be most useful if done iteratively, insofar as user feedback is gathered and modifications are made to the intervention numerous times. Hence, both the adaption of Baby Milk resources and co-creation of novel resources was done iteratively with continual input from the PPIE panel of parents. Moreover, the PBA offers a systematic method through which to integrate iterative and comprehensive target user feedback into the co creation process - rapid tabulation. Rapid tabulation involves using a Table of Changes (TOC) to systematically record all PPIE feedback. Thereafter, a process of agreement takes place between researchers to decide whether to adopt a piece of feedback or suggested modification in line with the intervention's guiding principles. This process uses the MoSCoW framework, whereby agreements can be made using the criteria: Must do / Should do / Could so / Would like to do (MoSCoW).[209] This allows intervention amendments and decisions to align with the overarching goals of the intervention, and keeps a detailed record of a fast evolving intervention prototype. Finally, key to the PBA is the generation of a theory of change to illustrate how the intervention seeks to achieve the desired behaviour change. As such, a theory of change model was also produced alongside co-creation, using the BCW for intervention design[202,209], which was iteratively modified throughout the further stages of intervention development.

Stage Two of the PBA: Optimisation

The second stage of the PBA - *optimisation* – seeks to optimise and evaluate the implementation of a designed intervention using mixed-methods approaches to ensure that to make sure that the resulting intervention is as meaningful, engaging, and useful as possible. Optimisation commonly involves 'real time user studies' where an intervention is tested and optimised in practice. Hence, the aim of our optimisation phase was to understand how users interact with the BRIGHT prototype and how they implement the resources in a real-world context, which might vary from when they are providing feedback on isolated sections of the contexts with a researcher. This can help to address the pervasive challenge of long-term participant retention and engagement within digital health interventions.[211,213] Whilst digital health interventions are often shown to be highly acceptable, they often see significant 'drop off' rates and uptake when implemented in the real world.[219] To counter this issue, the PBA proposes that real-time user studies can help to anticipate barriers to sustained engagement or uptake that emerge in the 'real world' and are therefore strongly encouraged for the development of novel digital interventions.[213,220] Three main methods are proposed for real-time user studies by the PBA, which can be chosen from based on the needs of the researchers, i) qualitative process analyses, ii) quantitative process analyses, and iii) think-aloud interviews.[209] The first, qualitative process analyses, was adopted for the current optimisation phase instead of think-aloud interviews. While think aloud interviews seek to measure immediate reactions to the intervention, qualitative process studies seek to understand users' experiences of the intervention over a period and access their attempts to change behaviours in a real-world context. [221]As BRIGHT will be delivered across the first year of life, evaluating sustained engagement in a real-world context and optimising the prototype for this purpose was particularly important to us. Moreover, as we had already received extensive feedback from the PPIE panel on the resources, we deemed 'immediate' think aloud feedback as less critical. For this reason, we undertook a qualitative process analysis, or as we refer to it an 'in the wild study', to evaluate engagement with the prototype of BRIGHT from target users in a real-world context. In this approach, users can try out the intervention in their own time and be retrospectively interviewed to collect input and optimise the design and delivery of the prototype. These interviews undertaken for BRIGHT were designed in line with the APEASE framework[222] – a theory-based framework to explore the prototype's; Acceptability (A), Practicability (P), Effectiveness (E), Affordability (A), Side-effects (S), and Equity (E). These methods are described fully in section 6.3.2.2.

6.3.2.2 The Behaviour Change Wheel (BCW)

It is crucial to note that the PBA described above does not seek to replace theory-based approaches to intervention development.[209] Rather, behaviour change theories and models – such as the Behaviour Change Wheel (BCW) – should be considered *within* the PBA process to ground interventions in a theoretical understanding of the behaviours targeted. Public Health Interventions seeking to change health-behaviours have historically produced modest effects, which in recent years has been ascribed to their lack of grounding in the theoretical understanding of target behaviours.[223,224] Hence, theory-based approaches to interventions seek to develop intervention components and strategies based on a theoretical understanding of the behaviours they seek to change.[225] This allows for determinants of behaviour to be systematically identified and then targeted to enable greater opportunities for behaviour change and intervention success. For example, the BCW can help identify relevant frameworks and behaviour change techniques which are relevant to the interventions target behaviours and then integrate them into interventions such as BRIGHT. There is an emerging evidence base demonstrating that interventions are more likely to effectively change the behaviours if they are designed in such a theory-led manner[218,219,222] using behavioural frameworks such as the BCW.[226] The BCW is an established comprehensive tool for the development of behaviour change interventions. Since the BCW's inception it has progressively been building a comprehensive taxonomy of target behaviours and effective Behaviour Change Techniques (BCTs) for use in population health behaviour change interventions.[227] Moreover, the authors of the BCW note that whilst many interventions may be 'theory-based', some theories of health behaviours – such as the Theory of Planned Behaviour or the Health Belief Model – do not cover the full range of behavioural drivers, such as habit formation. Moreover, there exists a large number of 'theory-based' interventions which are only minimally guided by relevant theoretical components.[225] What sets the BCW apart is that it assembles 19 individual frameworks of behaviour change, which alone may be useful at addressing some drivers of behaviour yet neglect others. Therefore, the BCW was adopted as it offers both a systematic and comprehensive method to guide the theoretical underpinnings of BRIGHT. Moreover, as Baby Milk was developed in line with the COM-B model[163], it's adoption for BRIGHT would aid consistency and comparability.

In the 'behavioural system' of the BCW – sources of behaviour are organised into three components represented by the COM-B model as illustrated in Figure 6.5.[202] The BCW proposed that for behaviour change to occur and be sustained, these three sources of behaviour need to be addressed. First, Capability (C) is a person's physical and psychological ability to adopt or engage with the target behaviour – such as having the resources and physical capacity to attend antenatal appointments in the first year of a child's life. Second, Opportunity (O) describes the factors outside of an individual – such as owning a smart phone - that influence a person's opportunity to interaction with a target behaviour or intervention. Finally, Motivation (M) captures the psychological processes which lead a person to feel motivated to engage with the target behaviour – such as feelings of distress that may lead to reduced feelings of motivation or self-efficacy. Within the COM-B model, each of these sources of behaviour interact with one another to influence behaviours and behaviour change. A given intervention may seek to target one or more of these sources of behaviour. COM-B unpacks the 'black box' of behaviour change – by considering what components of behaviour might be best targeted to successfully achieve behaviour change. For example, considering the behaviour of learning to brush one's teeth as a child, this behaviour is more successfully promoted if parents promote their child's capability (e.g., teaching the child to brush their teeth) and increase their motivation (e.g., by making the experience enjoyable by involving songs) to engage in the behaviour. The COM-B model thereby provides a useful framework to understand complex influences on behaviours that may be targeted in behaviour-change interventions.

Figure 1

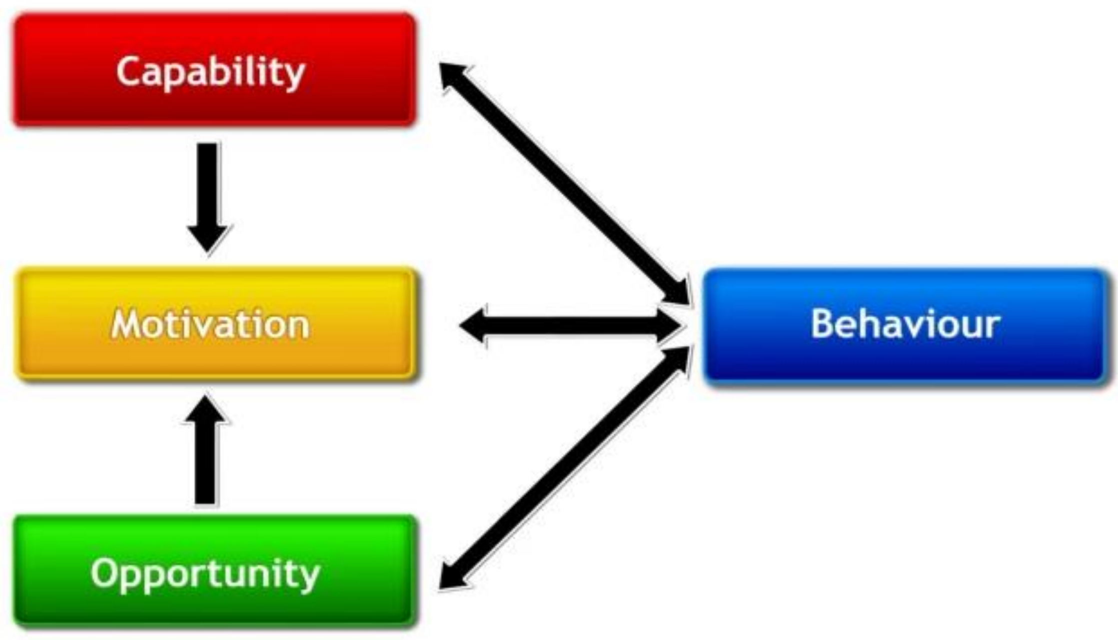


Figure 6.5. The Com-B System – a framework for understanding behaviour under the Behaviour Change Wheel obtained from Michie et al[202]

Behaviour Change Techniques (BCTs)

As part of the COM-B Model, Behaviour Change Techniques (BCTs) offer a shared language to describe what strategies might work to promote capability, opportunity, or motivation towards a desired behaviour.[226] Hence, BCTs are well-defined, observable, and replicable components of interventions seeking to target sources of behaviours. At present, the BCT Taxonomy (V1) consists of 93 hierarchically clustered techniques, which can be used in the development of behaviour change interventions.[227] BCTs allow researchers to explore which 'active ingredients' may be effective in changing relevant target behaviours, considering a wider array of BCTs than they might have adopted previously. Moreover, the BCT taxonomy is an imperative tool to tackle the 'black box' of intervention success, as it allows researchers to identify *which* elements of intervention were successful in changing behaviours and what might work well for other interventions. For BRIGHT, both the BCT taxonomy and the BCW was used across the three stages of intervention development. First, the BCT taxonomy will be used to map and understand the BCTs deployment in the Baby Milk Study from which BRIGHT is being adapted. This helped the research team understand what BCTs are implemented and can be brought forward, and which novel BCTs may be useful to implement in BRIGHT. For example, Baby Milk included; motivational components such as action planning, goal setting and self-monitoring, as well as coping planning to help parents deal with difficult situations.[163] For a full list of BCTs included in Baby Milk see Appendix X. Second, the BCW was also used to build a theory of change model of the BRIGHT resources. This ensured that the BRIGHT resources are co-created to address the sources of behaviour across the COM-B Model, as well as barriers and facilitators to appropriate and responsive feeding highlighted by the literature review and targeted in Baby Milk. Moreover, key behavioural targets were defined in line with the common language of the BCW to help with comparison to previous interventions and possible future interventions. Finally, the theory of change illustrated how the intervention design choices (e.g. the provision of a formula feeding tracker) will seek to achieve behaviour change and the long-term outcomes of BRIGHT. Taken together, utilising the BCW in these ways will help to ensure the development of BRIGHT is theory-led. Moreover, the detailed reporting of BCTs adopted will enable future research to further explore which active ingredients of interventions may be effective in improving infant growth outcomes and responsive feeding from the start of life.

6.3.2.3 The APEASE Framework

In stage three of BRIGHT's development, we undertook a qualitative 'in the wild' study (as a qualitative process analyses[209]) to evaluate and optimise a prototype of BRIGHTs formula feeding resources. In this evaluation, we wanted to ensure our optimisation and evaluation of the prototype was rooted in a theoretical understanding of components necessary to improve intervention engagement and change target behaviours. The APEASE framework, developed under the BCW, is a well-established and systematic method to investigate an interventions acceptability, practicability, effectiveness, affordability, side-effects and safety, and equity – each described in **Figure 6.6**.[202,222] Whilst APEASE offers a systematic approach to evaluation of interventions – it can be applied flexibly across varying stages of intervention development.[210,228] For instance, in earlier stages of intervention development it can be applied to make systematic decisions about which BCTs and intervention components to include. Whilst, in later stages it can be used to evaluate or optimise the delivery of an intervention. Moreover, the APEASE criteria can be assessed through numerous study designs - from trial designs to qualitative research. It may also be particularly useful to apply numerous designs to access each of the APEASE criteria. For example, whilst it may be useful to apply economic survey designs to access the affordability of an intervention, it is important to ask families themselves whether they may be able to afford implementing the components of an intervention in their day-to-day lives through qualitative methods. For the purposes of BRIGHT we used qualitative methods to access the APEASE criteria – using in-depth 1:1 interviews as part of our 'in the wild' study. As researchers have historically focused mostly on 'effectiveness' as a key intervention outcome, other components of interventions which can be equally important in achieving intervention success have been somewhat neglected.[213,222] For instance, although an intervention, such as face-to-face health visiting support for caregivers of infants, may be effective at promoting more frequent weight measurements, it may not be practical or affordable under the current healthcare system. For this reason, it is imperative to consider criteria such as equity as a key outcome, as families from varying socioeconomic backgrounds may differ in their abilities to access interventions.

APEASE Criteria	Description
Acceptability	How far is it acceptable to all key users?
Practicability	Can it be implemented as designed within the intended context, material, and human resources?
Effectiveness	How effective and cost-effective is it in achieving desired objectives in the target population?
Affordability	How far can it be afforded when delivered at the scale intended?
Side-effects and Safety	How far does it lead to unintended adverse or beneficial outcomes?
Equity	How far does it increase or decrease differences between advantaged and disadvantaged sectors of society?

Figure 6.6. APEASE criteria used to evaluate the BRIGHT prototype obtained from Michie et al[202]

6.3.3 Methodology of The Three Phases

6.3.3.1 Phase 1: Planning

6.3.3.1.1 Rapid Scoping Review of Qualitative Research on barriers and facilitators to adhering to formula feeding volume guidelines from existing literature

Following the decision to develop BRIGHT for caregivers of formula fed infants, it was evident that supporting caregivers to offer formula milk in line with the WHO formula feeding guidelines, would be a key aim of BRIGHT. However, no suitable systematic review summarising literature on barriers and facilitators to following formula feeding guidelines was available. Far more literature focused on alternative milk feeding behaviours – such as responsive feeding – or feeding in later childhood.[182] Therefore, a rapid literature review, led by Isabel Richie (an intern on the project), was undertaken to identify relevant barriers and facilitators to caregivers following formula feeding guidelines. The key aim of this rapid review was not to produce a comprehensive literature review for publication, but to identify the key barriers and facilitators to formula feeding behaviour to inform the design and identify relevant BCTs for BRIGHT. Moreover, as a systematic review of responsive feeding behaviours in infancy was identified[182], we planned to use key insights from both in the development of BRIGHT. Overall, key themes relevant to the barriers and facilitators were extracted from each of these reviews. The procedures undertaken to produce this scoping review are outlined in Appendix XI.

6.3.3.1.2 Behavioural coding of Baby Milk and Baby Buddy resources using BCTs to map and integrate existing intervention materials

To begin developing the BRIGHT intervention content, it was necessary to map out existing intervention materials to identify useful resources and key gaps. The BRIGHT research team first undertook a BCT mapping exercise to identify and code the BCTs used within the Baby Milk materials which would eventually be translated into BRIGHT. All Baby Milk materials – the baseline visit protocol, healthy growth and nutrition leaflet, personal feeding plan, stickers to cover formula manufacturers recommended amounts, follow up phone calls, and the facilitator training resources - were coded using the V1 BCT taxonomy[227] and in line with the COM-B functions ‘capability’, ‘opportunity’ or ‘motivation’. All materials were initially coded by two independent coders (KT and SE) and disagreements were resolved through discussion at several stages of the process. The full list of BCTs utilised in Baby Milk is presented in Appendix VI.

6.3.3.1.3 Development of a BRIGHT user journey

BRIGHT was conceptualised to be a multi-component intervention with scope to personalise the content a caregiver receives based on individual characteristics of the baby, caregiver needs and circumstances, and other information added to the app. We developed the BRIGHT user journey to envisage the key elements of BRIGHT and how users would be introduced to, and moved through, the intervention. Moreover, we drafted a structure of how BRIGHT would be delivered within the existing framework and resources of the Baby Buddy app. This helped identify where more resources needed to be produced through later co-creation, as well as where interactive features and pre-existing algorithms in Baby Buddy’s digital architecture could be utilised. This user journey was updated and refined throughout the BRIGHT development process in line with stakeholder and PPIE feedback. The development of the BRIGHT videos is described in Appendix XII. Finally, in as part of this exercise the research team began to develop BRIGHTS guiding principles as described below in section 6.4.1.1. These guiding principles for BRIGHT were initially developed between the research team and the Baby Buddy team, and then further refined using user feedback from the PPIE panel in line with the PBA approach.

6.3.3.2 Phase 2: Co-Creation

6.3.3.2.1 Design

In line with the PBA, a PPIE panel was used to co-develop resources with the intended user population through a pragmatic and iterative process.[209,214] We recruited a panel of caregivers that would offer feedback on the evolving BRIGHT content to facilitate this process of co-creation. BRIGHT therefore benefitted from feedback at multiple timepoints - as and when needed. Moreover, BRIGHT formula feeding content was adapted from the established Baby Milk trial materials which had been developed using extensive qualitative methods to understand formula feeding experiences in the first year of life.[171] This was another avenue though which qualitative input shaped the materials.

In this phase of development, A Theory of Change Model was also produced for the formula feeding and growth resources to: i) define the behaviours and sub-behaviours which BRIGHT seeks to change; ii) identify barriers and facilitators to these behaviours using previous literature; and iii) identify which behaviour change techniques can be used in BRIGHT to achieve this behaviour change – as highlighted in **Figure 6.7**. Hence, this exercise aimed to define the behaviours BRIGHT is addressing and how it seeks to address them. This exercise was undertaken following the PPIE stage of co-creation and was led by Zohar Preminger with supervision from myself. The full procedure for developing this theory of change model is presented in Appendix XIII.

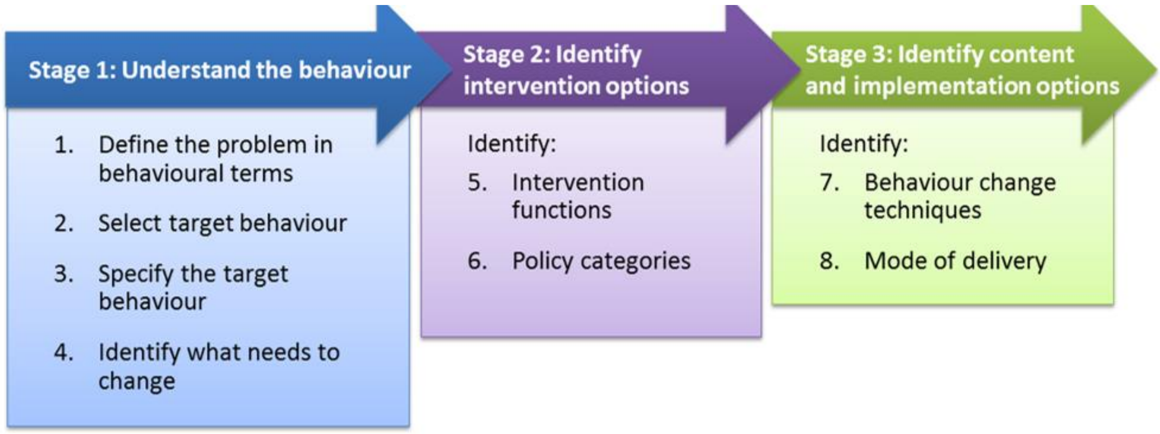


Figure 6.7. Diagram outlining the process of the behaviour change wheel (BCW) obtained from Michie et al[202]

6.3.3.2.2 Participants / PPIE

For the PPIE panel, we recruited caregivers who lived in the UK, were exclusively or primarily formula feeding their infant (or had done so prior to the introduction of solid foods) and had a baby between the ages of 6-12 months old at the time of recruitment. We refreshed the PPIE panel with new members at later stages of intervention development where PPIE panellists had dropped out, and as existing PPIE panel members' babies got older, using the same criteria. For the growth, appetite, and complementary feeding modules PPIE panel inclusion criteria were expanded to include caregivers who were also breastfeeding, as these resources would be applicable for them as well. To ensure BRIGHT content was acceptable and appropriate to Baby Buddy's demographically diverse user population and developed in line with *proportionate universalism*, we targeted recruitment efforts towards caregivers from diverse backgrounds – representing fathers, single caregivers, parents with English as a second language and families living in economic hardship.[208]

6.3.3.2.3 Procedure

Participants were recruited through multiple channels including the Lambeth Early Action Partnership (LEAP) Coffee and Chat sessions, Social Media advertisements (e.g. Facebook groups for formula-feeding parents), existing networks of Baby Buddy users, and existing UCL-based research networks of parents willing to participate in research. Once contacted, parents were asked to fill out the participant information and demographic sheet to provide demographic information, details of their infant feeding practices. Participation in the PPIE panel was compensated with a £25 online Love2Shop shopping voucher (1 per interview or round of written feedback), to thank participants. Reimbursements were in line with the NIHR INVOLVE guideline for fair compensation of the public in health research.

The PPIE feedback took place in an iterative approach in line with the PBA, asking for caregiver feedback as the BRIGHT materials were being drafted. PPIE feedback therefore consisted of several rounds, with each BRIGHT article, video script, or interactive feature gathering feedback from at least 5 caregivers. Once again, where an insufficient number of caregivers responded to calls for feedback in the later rounds of PPIE, new caregivers, meeting the same criteria, were recruited. PPIE feedback was provided through either a virtual interview or through written feedback sent over email. Any virtual interview sessions were transcribed verbatim using the Microsoft Teams transcription service, anonymised, and cleaned following the session. The full PPIE documents for BRIGHTS formula feeding resources are presented in Appendix XIV.

6.3.3.2.4 Analyses

After collecting the feedback, quotes and written feedback were organized using Rapid Tabulation, in line with the PBA. Rapid tabulation involves using a Table of Changes (TOC) to systematically record all feedback and used the MoSCoW framework to decide whether suggestions would be implemented.[209,221] Any disagreements were discussed prior to coming to a final consensus. Where parental feedback contradicted a key principle of BRIGHT or contradicted infant feeding guidelines, they may have been deemed inappropriate to integrate. All changes have been recorded in the Rapid Tabulation Document presented in Appendix XV. Moreover, the PPIE panels feedback on the BRIGHT resources were later revisited in order to generate overarching themes that reflected the caregivers, who participated in either BRIGHTS PPIE panel of qualitative study, reflections and experiences as formula feeding caregivers. This is detailed in section 6.3.3.4.

6.3.3.3 Phase 3: Optimisation

6.3.3.3.1 Design

The third stage sought to test the BRIGHT prototype in a real-world setting using. We used an 'in the wild' qualitative study – a type of Qualitative Process Analyses as part of the PBA – to understand how the BRIGHT formula feeding modules engaged target users in a real world context and over an extended period of time.[209,217] Interviews sought to specifically assess potential behaviour change for BRIGHT's four target feeding behaviours against the APEASE criteria: i) choosing a suitable formula milk; ii) preparing a bottle of formula milk safely; iii) providing an age-appropriate quantity of formula milk; iii) bottle-feeding responsively. These target behaviours were informed by the theory of change model developed under Phase 2.

First, we developed an interactive prototype of BRIGHT formula feeding modules on Canva for this stage of research.[229] Canva is an online graphic design platform which can be used to design semi-interactive app prototypes (see Figure 6.8). This first prototype included the formula feeding, growth monitoring, sleep, and crying modules, as well as the personalized growth chart feedback. The prototype did not include the BRIGHT videos, as they had previously received extensive PPIE feedback and input and had been reviewed and signed off by the Baby Buddy editorial board before filming began (in Stage 2) – they could not therefore be changed or 'optimised' as the written content could. Moreover, the appetite module and quizzes were tested separately through 'think aloud' interviews and, as such, were not included in the 'in the wild' study. Overall, the 'clickable' prototype of the formula feeding resources were designed to emulate the Baby Buddy app design using Canva. The full prototype can be accessed in Appendix XVI.

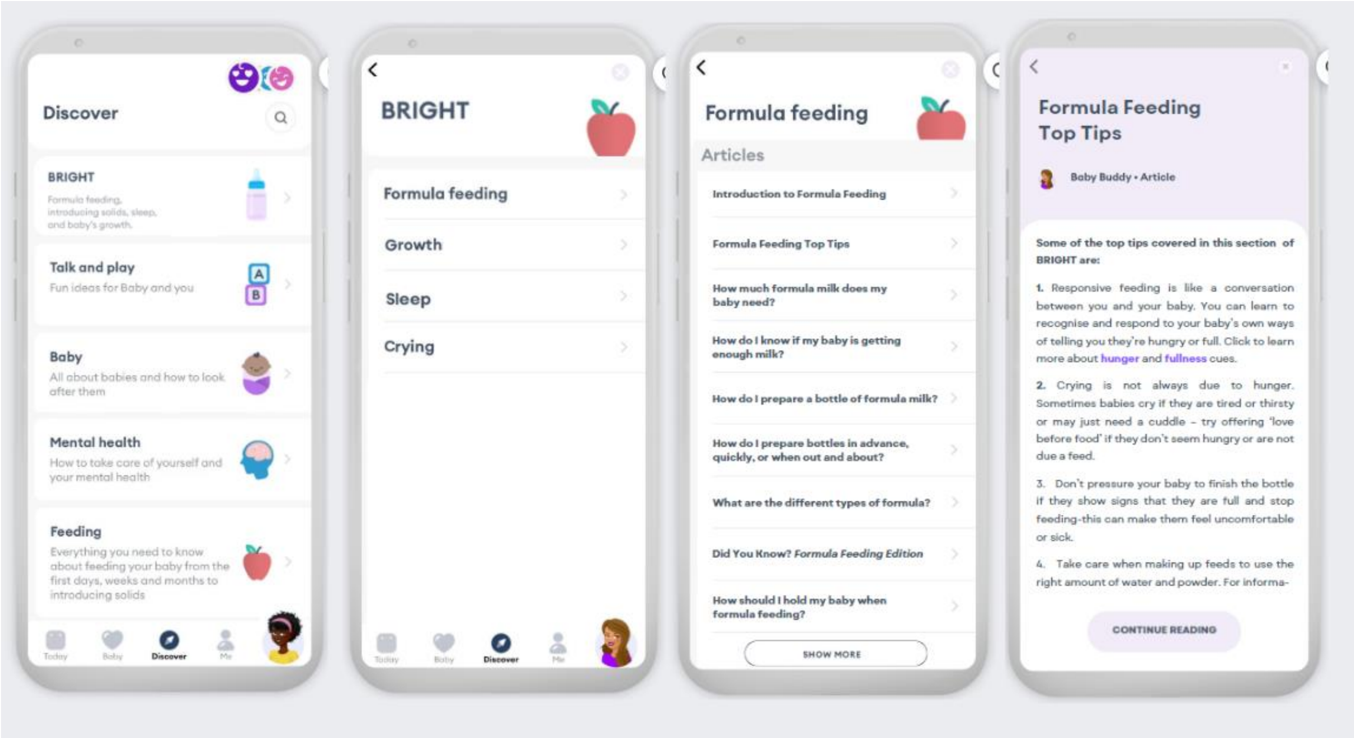


Figure 6.8. Examples of prototype screens. From left to right; Baby Buddy Homepage, BRIGHT landing page, Formula feeding module page, Article on Formula Feeding Top Tips

'In the wild' user-testing is a qualitative process evaluation tool for digital interventions recommended as part of the PBA.[209] This method allows researchers to understand how users experience the intervention as a functional prototype, and how intervention components may be adopted or disregarded in the context of the users' day-to-day lives. Hence, the goal is not to collect immediate feedback or reflections about how other individuals might engage with the intervention – such as with think aloud interviews - but a reflective summary of how the participants themselves engaged with the intervention over an extended period. For the 'in the wild' study, a subset of Baby Buddy Users (n=7) were provided with access to the prototype for 2-weeks, and were asked to use the intervention as they wished to throughout their day-to-day activities. These users had not been previously involved in any BRIGHT PPIE research. We sought to recruit around 5 caregivers, given the relatively high demand of participation and extensive PPIE co-creation that had already been undertaken on the resources previously. Moreover, as we were not seeking to make representative inferences about caregiver's experiences, but instead to capture target users experiences of the BRIGHT prototype, we did not feel it critical to undertake this phase of research with a much larger sample. Following this 2-week period, each participant took part in an online semi-structured 1-2 hour interview, with Zohar Preminger, to explore their experiences of using the prototype in the context of their daily lives, in line with the APEASE criteria.[210] Participants were encouraged to take notes of their experiences of BRIGHT throughout the 2-week period, however this was not instructed as necessary given the already high demands of participating on new parents.

6.3.3.3.2 Participants

Existing Baby Buddy users who were either exclusively or partially formula feeding an infant under the age of 6 months were invited to take part in the 'in the wild' study. Here, we allowed for partially formula fed infants – who were fed predominantly formula milk with some breastmilk supplementation – to facilitate a greater pool of eligible participants and as these resources would be relevant to their experiences as well. All users were not familiar with the BRIGHT project, and none had been involved in the previous PPIE research, as we sought to capture novel perspectives. Participants were recruited through a notification on the Baby Buddy app where they expressed interest in participating and completed a demographic questionnaire to ensure their eligibility.

6.3.3.3.3 Procedure

Once participants had indicated their interest and filled out the demographic questionnaire, eligible caregivers were sent an information sheet and further instructions on how to access and use the BRIGHT prototype over the two-week period. Each participant was then scheduled for a 60-minute interview via Microsoft Teams (Version 1.6.00.20074) or Zoom (Version 5.15.5 (19404). The interview explored participants' use of the prototype, tapping into the different APEASE criteria from the BCW. A semi-structured interview schedule, guided by the APEASE criteria, was developed and used for each interview. This interview guide included open questions framed around each component of the APEASE framework, and also provided participants with the opportunity to suggest further modifications that might improve the prototype. As suggested by the PBA, open questions were adopted for interviews to give participants freedom to tell their own story. See Appendix XVII for the full interview guide. The interview procedure was piloted by 4 members of the BRIGHT team and adjusted multiple times to ensure the questions were easy to understand and respond to, and that the length of the interview was appropriate.

6.3.3.3.4 Analyses

Interviews were recorded and transcribed using the Microsoft Teams and Zoom software, with transcripts anonymised and video files deleted. Transcripts were uploaded into NVivo® (Version 14, March 2023)[230,231]. and coded inductively and deductively using Braun and Clarke's guide to reflexive thematic analysis (RTA).[232] The derived codes were then grouped into the corresponding APEASE criteria in line with the aims of the study. Following the interview coding process, modifications that were suggested by participants were recorded and reviewed by the BRIGHT team. Once again, they were adopted into the BRIGHT prototype if in line with BRIGHTs guiding principles. All modifications suggested by participants are recorded in Appendix XVIII for reference.

6.3.3.4 Overarching Themes from PPIE and In the Wild Interviews

As a final synthesis stage of analysis, data from both the PPIE feedback and the 'in the wild' interviews were reviewed to highlight relevant and novel themes regarding the formula feeding, sleep, and crying resources of BRIGHT. These themes seek to represent the key insights gathered from caregivers under the BRIGHT development which we see important to highlight to guide future interventions and research in this field. The themes represent caregiver perceptions of the BRIGHT prototype, their experiences in implementing BRIGHT, as well as their previous experiences using a digital technology for infant feeding. These insights will be used to further guide the evaluation stages of BRIGHT, and may be used in future research and interventions that target the infancy period. To generate these themes, I revisited the PPIE feedback rapid tabulation documents, 'In the wild' interview thematic analysis, and quotes on the final prototype. These themes were discussed amongst the research team and modified accordingly.

6.3.3.5 Statement of Reflexivity

Overall, the BRIGHT research team includes diverse members who consist of both parents and researchers without children. The core BRIGHT content was originally produced by Kristiane, who is European white, not a parent or caregiver, experienced in using technology, and would be considered as having a high socio-economic background and postgraduate level education. Moreover, the wider research team would also be considered as having a high socio-economic background and education level consisting of mainly academic researchers, although the team are more ethnically diverse, including both White and Asian-British researchers. Nonetheless, the research team placed significant emphasis on mitigating their inherent biases, due to their backgrounds and experiences, by including a diverse set of caregivers in the PPIE panel. Moreover, as Best Beginnings places significant importance on providing representative and accessible resources, their content team with substantial experience were consulted on multiple occasions to help improve the accessibility of BRIGHT. Finally, to mitigate the impact of researcher bias within the 'in the wild' qualitative study a semi-structured interview guide was followed and a separate researcher from the PPIE phase, Zohar Preminger, led on conducting the interviews. Nonetheless, it must be taken into consideration how the research team's backgrounds and characteristics may have influenced the production of the resources, and the data collection and analysis.

6.4 Results

6.4.1 Phase 1: Planning

6.4.1.1 Guiding Principles for BRIGHT Intervention

In line with the PBA, in collaboration with the wider research team and Best Beginnings, five guiding principles were defined as described in the figure below. These were developed by the research team, in collaboration with our charity partners Best Beginnings, and informed by the ongoing PPIE feedback. Moreover, these we're refined on an ongoing basis in line with the iterative PPIE process and fast-evolving nature of digital health intervention development.

Guiding Principles for BRIGHT Intervention

1. Prevent overfeeding, with both formula milk and the introduction of solid foods during the first two years of life
2. Support responsive parenting, with a focus on responsive feeding.
3. Acknowledge individual differences in infants and children (e.g. in appetite, growth, sleep, crying/temperament)
4. Ensure language is accessible, empathic, supportive, non-judgemental, non-didactic
5. Offer practical and realistic advice and strategies that work for families living in a range of circumstances

Figure 6.9. Guiding Principles for BRIGHT Intervention

6.4.1.2 Rapid Scoping Review of Barriers and Facilitators to caregivers following formula feeding guidelines

Overall, the rapid review identified nine qualitative studies exploring barriers and facilitators to adherence to formula feeding guidelines.[151,163,172,175,233–237] Overall, these studies yielded far more barriers to following formula feeding guidelines than facilitators as shown in **Table 6.1**.

Facilitators

Only one study, Lakshman et al[24] presented a facilitator to following formula feeding guidelines. The Baby Milk study highlighted that **promoting caregiver self-efficacy** in adhering to guidelines by increasing motivation, offering action planning, and coping planning, was welcomed and potentially a key intervention component that led to reduced overfeeding in the trial evaluation.

Barriers

Five studies highlighted a theme of **missing and mixed messaging** in formula feeding guidance and resources.[233–237] These gaps were reported on a range of topics such as safe formula feeding preparation, how much to feed, how to feed in response to hunger cues, as well as scarce antenatal support for formula feeding. For instance, Lagan et al's focus groups highlighted that mother had received varying best practice from differing health care professionals regarding formula feeding guidance, leading to confusion.[234] Moreover, this study highlighted maternal perceptions of a lack of formula feeding guidance, which may be due to the persistent societal promotion of breastfeeding, and the concern among healthcare professionals about undermining it by providing information about formula feeding.

Secondly, several studies highlighted a **lack of support from the healthcare system** around formula feeding as a clear theme.[144,151,235] This theme was particularly apparent in Kotowski's focus groups with nurses, where they reported a lack of confidence in providing bottle-feeding guidance, as well as difficultly in their ability to share bottle-feeding advice while following national breastfeeding guidelines.[151] Some highlighted **the scarce formula feeding guidance available from reputable sources**, thereby leaving parents to turn to less reputable sources for guidance, such as websites and apps from formula milk companies.[144,175,235] One study raised the theme of the **low accessibility of formula feeding resources** such that information written on formula milk packs and guidance tended to be too complex, and requiring too high a level of health literacy for many caregivers.[236] This limitation may be exacerbated by caregivers' necessary reliance on formula packs for guidance on formula feeding amounts, given the lack of resources about formula feeding from healthcare and other professional organisations.

Table 6.1. Barriers and facilitators to adhering to Formula Feeding Guidelines Identified by Rapid Review

Theme	Actionable information for BRIGHT Intervention Development	References
Facilitator		
Promote caregiver self-efficacy	• Include resources that increase caregiver motivation, coping planning and confidence in following formula feeding guidelines • Utilise digital delivery to include prompt reminders	Lakshman et al (2015)[65]
Barriers		
Missing and mixed messaging	• Include information on the commonly unaddressed areas in existing resources: safe preparation; how much to feed; how to feed responsively • Ensure information is clear and consistent • Provide comprehensive information in line with best practice guidelines to avoid the need to for users to turn to other sources which may give conflicting or incorrect advice	Tarrant et al (2013)[233] Lagan et al (2014)[234] Russel et al (2016)[235] Gilmore et al (2020)[236] Chourqui et al (2019)[237]
Lack of support from healthcare system	• Help to decrease perceptions of healthcare workers as inaccessible for formula feeding advice (increase caregiver motivation to ask for help) • Ensure caregivers are informed about how to access support from healthcare professionals (both routine checks and additional contact) • Acknowledge healthcare provider difficulty in sharing information on formula feeding whilst respecting national breastfeeding guidelines • Fill this gap in provision with sufficient advice for caregivers	Appleton et al. (2018)[172] Russell et al. (2016)[235] Kotowski et al. (2021)[151]
Scarce formula feeding guidance from reputable sources	• Highlight the dangers of rigidly following pack guidance when formula feeding and need to recognize hunger cues • Increase accessibility of professional guidelines in a digital format	Russell et al. (2016)[235] Appleton et al. (2018)[172] Appleton et al (2019)[175]
Low accessibility of feeding guidance	• Be written below college (university) reading level to be accessible to the general population, and therefore effectively transmit information	Gilmore et al. (2020)[236]

The BRIGHT intervention seeks to address each of these identified barriers by co-developing formula feeding resources with caregivers, which are comprehensive, clear, and avoid mixed messaging. Moreover, BRIGHT will indicate how support from healthcare workers may be accessed and ensure a high accessibility – developing the resources to be of a reading age of ~12 years with help from Best Beginnings' Content team.

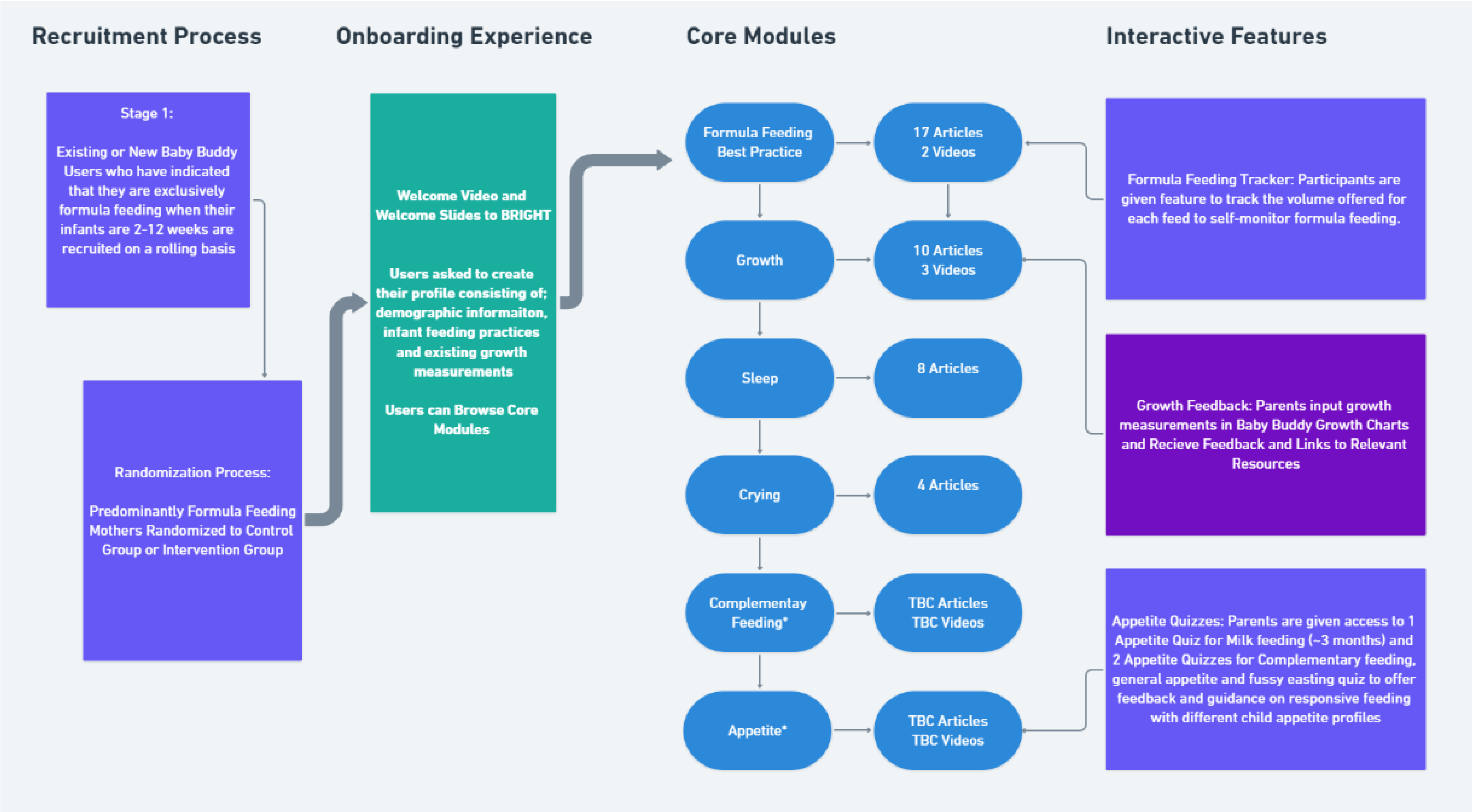
Behavioural Coding of Baby Milk

All Baby Milk intervention materials were accessed and coded in line with the COM-B Model's taxonomy of 93 BCTs.[227] For the full BCT coding table see Appendix X. Across the 6 resources of Baby Milk, 31 BCTs were identified. Behavioural coding of the Baby Milk materials also highlighted key gaps in the existing resources, which were identified for expansion in BRIGHT. The key areas of guidance not covered in Baby Milk included: i) responsive bottle feeding – recognising and responding to infant hunger and satiety cues and responsive bottle positioning; and ii) understanding and responding to infant appetite. It was also noted that Baby milk provided little guidance on sleep or crying behaviours related to feeding – for example how to soothe a crying baby without using a feed if they are not demonstrating hunger cues, and how to support infants in establishing healthy sleeping routines, which don't involve feeding them to sleep. We deemed these aspects to be important to include for a complete 'formula feeding package' in BRIGHT and they were therefore developed independently by the BRIGHT team, with input from Professor Ian St-James Roberts, a child psychologist with expertise in supporting parents to manage infant sleep and crying.

6.4.1.3 Development of the BRIGHT Intervention User Journey

A user journey wireframe was developed and continuously updated to envisage how the BRIGHT prototype would be dynamically implemented through the Baby Buddy app. It also neatly summarises: (i) the six core modules of articles and videos on; Formula Feeding, Crying, Sleep, Growth Monitoring, Appetite (not detailed in this chapter), and Complementary Feeding (not detailed in this chapter); (ii) the three interactive intervention components - Digital Growth tracking with tailored weight feedback, Appetite quizzes with tailored feeding feedback, and a Formula feeding tracker with tailored feeding feedback;

and (iii) how the different components link up. Initially, it was envisioned that BRIGHT would involve weekly in-app notifications to promote engagement with the resources. However, given both financial and technical constraints, a bespoke notification pathway for BRIGHT was not possible under the initial stage of development described in this thesis. Possibilities for bespoke BRIGHT notifications are currently being discussed with Best Beginnings, to inform the funding we may need to seek to enable the development of such a feature.



*Complementary feeding and Appetite not included in the present thesis and will be co-created / optimised in future stages of development

Figure 6.10. Prototyped Wireframe for BRIGHT Trial Participant User Journey

As Baby Buddy places significant emphasis on offering caregivers short informational videos on various parenting behaviours, a proportion of resource was used to develop videos on formula feeding, growth monitoring, and complementary feeding. In this way, BRIGHT would align with the design and delivery approach of Baby Buddy. Analysis of the existing Baby Buddy content library confirmed that no video-format resources were available for using and interpreting growth charts in infancy, which are often subject to confusion due to their inherently technical nature.

Moreover, infants vary significantly in their temperament[238], appetite[239], and growth[22] right from the start of life, meaning parents experience varying challenges in early feeding experiences. The digital format of BRIGHT provides novel technology that can enable feeding guidance to be tailored to these unique challenges. Hence, BRIGHT sought to integrate tailored components, informed by expertise from the research team as well technical input from Best Beginnings and feedback from the PPIE panel. Three areas of the intervention lent themselves to incorporate a tailored element: (i) growth monitoring, (ii) tracking of formula milk intake and (iii) infant appetite. Efforts focused primarily on the development of a *growth feedback* feature, as it fell within the remit of existing development work for the charity and was affordable to build. Minimal prototype versions of the two other features - the Appetite Quizzes and the Formula Feeding Tracker - were produced and will be developed once additional funding is secured for the later stages of BRIGHT evaluation. A description of each 'interactive feature' of BRIGHT is included in the next section. A description of the Appetite Quizzes and Formula Feeding Tracker are provided in Appendix XVIX.

Tailored Growth Feedback

Growth monitoring is a routine and key aspect of infant care, which allows parents to track the health and development of their baby.[141] At present, caregivers can manually monitor their infant's growth using the UK-WHO growth charts provided to parents in their physical NHS Red Book.[141] However, parents often report difficulty in interpreting growth charts, and understanding how or if this should information should be used to modify their feeding practices.[240,241]

A digital version of these growth charts is currently embedded in the Baby Buddy, allowing users to manually add their baby's height and weight data. This visually maps the infant's age- and sex-adjusted weight percentiles across the period of recording. BRIGHT aimed to provide caregivers with actionable feedback on their infant's current weight centile, in response to inputting any weight data. Varying personalised feedback messages would be received for each of the following centile bands: >=91st, 75th-91st, 9th – 75th, <9th and <2nd centile spaces. Accompanying information would then parents with guidance on how to interpret their child's current weight centile, in the context of previous measurements (i.e. their weight gain trajectory). For instance, if their infant has a high weight centile (e.g. 91st), and they appear to have moved from a much lower centile previously, they are provided with links to guidance on responsive formula feeding, common challenges when feeding (which highlights tips to avoid overfeeding), and tips for feeding a hungrier baby. This personalisation of feedback nudges them towards relevant feeding guidance. These feedback messages were refined with a subsample of the PPIE panel (n=6 parents). These results are reported in section 6.4.2.3 below.

6.4.2 Phase 2: Co-Creation

A PPIE panel was utilised to co-create the development of the following BRIGHT resources:

1. Five video scripts [formula feeding, growth monitoring, weaning, complementary feeding, and a 'Welcome to BRIGHT' video]
2. Formula feeding module [17 articles]
3. Growth monitoring module [10 articles]
4. Sleep module [8 articles]
5. Crying module [4 articles]
6. Tailored Growth Feedback

6.4.2.1 Descriptive statistics of the BRIGHT PPIE panel

Descriptive characteristics summarizing the family and infant characteristics of the entire PPIE panel are presented in **Table 6.2**. Overall, 15 caregivers were recruited, of whom most were mothers in addition to 3 (20%) fathers. Most participants were White (n=7, 40%), with the remaining caregivers being of Asian or Asian British (n=2, 13.3%), Mixed (n=3, 20%) as well as Black or Arab ethnicity (n=3, 20%). The sample was overall highly educated, with 8 (53.4%) parents having a postgraduate degree. Most of the panel members had used primarily formula milk to feed during the milk feeding period (80%), however some breastfeeding parents were included to review resources on growth monitoring, sleep and crying. Finally, most parents (86.7%) reported having previously used mobile applications to care for their infant or seek guidance on infant feeding.

Table 6.2. Demographics of BRIGHT PPIE Panel (n=15)

		PPIE Sample (n=15)	
		N	Mean or %
Age of Participant at Recruitment		15	39.6
Age of Child at Recruitment (in months)		15	24
Parental Relationship			
	Mother	12	80%
	Father	3	20%
Relationship Status			
	Long Term Relationship or Married	12	80%
	Single	3	20%
Ethnicity			
	White	7	40%
	Asian / Asian-British	2	13.3%
	Black/African/Caribbean/Black British	2	13.3%
	Arab	1	6.6%
	Mixed or Multiple Ethnicities	3	20%
Education Level			
	Postgraduate Qualification	8	53.4%
	Undergraduate Qualification	3	20%
	A or AS Level	1	6.6%
	Vocational Qualification	3	20%
Infant Feeding Method in Milk Feeding Period			
	Primarily Formula Feeding	12	80%
	Primarily Breastfeeding[a]	3	20%
Number of Children			
	One	9	60%
	Two	3	20%
	Three	0	0%
	Four or more	3	20%
Using Apps for Infant Feeding[b]			
	Yes	13	86.7%
	No	2	13.3%

PPIE; Patient and Public Involvement and Engagement
[a]Included for the co-development of sleep, crying, and growth materials
[b] Do you or have you used any parenting/feeding mobile apps since having your baby

6.4.2.2 PPIE Feedback and modifications to written BRIGHT materials

The full rapid tabulation of the key feedback across the intervention components is presented in Appendix XV. **Table 6.3** presents examples of this process, and how suggested modifications were applied to the prototype. Suggested modifications largely fell into three categories: modifying language to improve acceptability of the materials; adding statements to enhance accuracy of parents' experiences; and formatting modifications to improve the readability and engagement with the materials.

In regard to the tailored growth feedback specifically, parents found the information acceptable, and offered improvements to ensure the accessibility and acceptability of the language used. However, parents suggested additional explanation and reassurance when infants had a high or low weight centile, given the commonplace anxiety felt by parents in these situations. In response, we developed two additional FAQ articles ('What if my baby is on a high centile on the growth chart?' and 'What if my baby is on a low centile on the growth chart?') to further explain centile crossing, and point them towards responsive feeding guidance and guidance on age-appropriate volumes of formula milk. See Appendix XX for the growth feedback messages refined in line with the PPIE panel.

Table 6.3. Extract of Table of Changes Undertaken after PPIE Feedback

Example	Resource	Section	Comment	Suggested Change	Reason For Change and Guiding Principle	MoSCoW[a]
1	Formula Feeding Article: How do I Bottle Feed My Baby Responsively?	*Try to avoid distractions whilst feeding, such as phones or a TV playing. We understand you'll be feeding a lot, but it's important to keep your focus on baby so that you spot his/her signals of fullness*	I do find this point a little patronising as I think people do know to focus on their baby as best they can during feeding. But also people breast feed while watching TV all the time (and nobody really tells them to focus on their baby!). I do probably just remind that babies can sometimes be distracted by things going on when feeding i.e. tv, other people, etc so if the parent notices this is happening, it's a good idea to avoid those distractions and create a more calming environment.	Remove Patronizing Language: Try to create a calm environment for feeding: Babies can easily be distracted, especially as they get older, so avoiding distractions as much as possible whilst feeding is important. Creating a calm environment for feeding might be easier on some days than others, and that's okay. When it's possible, putting aside phones and minimising noise can help you and [Baby] bond, and can help you spot [their] fullness cues. We understand you'll be feeding a lot, but it's important to keep your focus on baby so that you spot his/her signals of fullness.	Improve Acceptability Ensure language is: accessible, empathic, supportive, non-judgemental, non-didactic	Must do

2	Growth Monitoring Article: What is my Baby's Weight Centile	*Weight centiles are useful to measure baby's growth, and you can learn more about what they are and how to use them in this article or in this video [Link to video].*	I think it would be helpful for the language to be a bit more emphathic at the beginning. I.e. 'its understandable and natural to notice other babies and wonder whether your baby is too small or too big compared to them, however….'. It just feels a little cold at the beginning.	Modify Article to Be More Empathetic: Added: - It is common to notice how other babies are growing in comparison to yours. But remember, each baby is different and understanding how your baby is growing on their growth chart can help reassure you that they are growing well or help spot whether they might need a bit of help'	Increase Engagement	Should Do
4	Crying Module: How much crying is normal?	Giving too much formula or bottle-feeding too quickly can trigger colic episodes or make it worse	this sounds very judgmental on parents who are not able / do not choose to breastfeed. We had breastfed babies in our pre-natal group that developed colic	Remove reference to bottle-feeding: Feeding your baby too quickly can trigger colic episodes or make it worse	Improve Acceptability	Must do
6	Sleep Module; How can my baby learn to fall asleep on their own?	Full article	I think maybe a caveat here that you don't have to teach babies to fall asleep on their own if you don't want to. Whilst it was important to me- once my baby showed me she could self settle- I know people who were happy to feed to sleep/rock to sleep/ safely co-sleep for quite some time.	Add statements to module that say that feeding decisions are highly personal: I.E This will help Baby to sleep for longer at night, because when s/he wakes up, s/he will know how to drift off to sleep again by him/herself. You might prefer to comfort or rock your baby to get to sleep regularly, this is your	Improve Acceptability	Must Do

		decision as parent. However, there are some steps you can take to help your baby learn to fall asleep on their own without needing comfort or a feed:	

[a]MoSCoW: Must do, Should do, Could do, and Would like.

6.4.2.3 Theory of Change

Following the BCW framework, the BRIGHT resources underwent a theory of change exercise. This exercise sought to map out and define the target behaviours and identify relevant functions and Behaviour Change Techniques (BCTs), which would be implemented in BRIGHT. These elements were presented in line with relevant barriers and facilitators to responsive feeding behaviours. In sum, a wide range of BCTs were implemented into the BRIGHT intervention, spanning 6 functions: education, modelling, persuasion, enablement, training, environmental restructuring. The procedure for this theory of change is presented in Appendix XIII. Several themes and insights emerged from this process and were discussed amongst the research team as described below. The full Theory of Change model and description is presented in Appendix XXI given its comprehensiveness and detail. An excerpt from the theory of change is however presented below – with one illustration of how BRIGHT targeted each of the COM-B functions; capability, opportunity and motivation. Overall, this comprehensive behavioural mapping process helped to identify which behavioural targets would be addressed (e.g. recognising an infant's unique appetite) and *how* (e.g. offering an appetite quiz) to achieve the intended long term-outcome of BRIGHT (e.g. increase responsive feeding behaviours – e.g. reduced pressured feeding) using the established BCT taxonomy. Characterizing the intervention in this manner is essential to facilitate future evaluation work.

Table 6.4. Excerpt from BRIGHT theory of Change Model

COM-B Component	**Barrier / Enabler to Responsive Formula Feeding**	**Barrier / Enabler to Appropriate Formula Feeding**	**Target sub-behaviour**	**Intervention Functions and Behaviour Change Techniques**	**Proposed Mediating Variables**	**Long-term Outcomes**
Capability	Enabler: Education and information	Barrier: Scarce formula feeding guidance from reputable sources	7. understanding the importance of responsive feeding 8. understanding the consequences of overfeeding 1. recognising the baby's hunger and fullness cues 4. not soothing the baby with a feed 15. understanding typical growth trajectories 16. Recognising Rapid Infant Weight Gain	**Education** 4.1 Instruction on how to perform the behaviour 4.2 Information about Antecedents 4.3 Re-attribution **Modelling:** 6.1. Demonstration of the behaviour 8.2 Behaviour Substitution **Persuasion:** 9.1 Credible source 15.1 Verbal persuasion about capability 5.1 Information about social and environmental consequences 5.6 Information about emotional consequences 1.1 Goal setting (behaviour) 1.4 Action Planning 1.5 Review Behavioural Goals	Promote Responsive Feeding Practices Promote caregiver recognition of hunger & satiety cues Prevent overfeeding Understand growth charts and growth trajectories Recognise Rapid Infant Weight Gain Promote Caregiver Self-Efficacy and Autonomy	Less Rapid Infant Weight Gain Improved Infant Appetite Regulation Promote Responsive Parental Feeding Habits and Behaviours

				1.8 Behavioural Contract 2.6 Feedback on Behaviour 2.3 Self-Monitoring of Behaviour 2.4 Self-Monitoring of Outcomes of Behaviour 2.7 Feedback on outcome(s) of behaviour **Enablement:** 8.4 Habit reversal 1.2 Problem solving 1.4 Action planning 7.1 Prompts/cues 7.5 Remove aversive stimulus 9.3. Comparative Imagining of future outcomes 11.Reduce negative emotions 2.6 Feedback on Behaviour 2.7 Feedback on outcome(s) of behaviour **Environmental restructuring** 12.1 Restructuring the physical environment 12.2 Restructuring the social environment 7.5 Remove aversive stimulus 12.5 Adding objects to the environment		

Opportunity	Barrier: maternal distraction to physical objects during feeding		10. putting aside distractions 11. focusing on the baby when feeding 12. choosing a quiet place to feed (when possible)	**Education:** 4.1 Instruction on how to perform the behaviour **Modelling:** 6.1. Demonstration of the behaviour **Persuasion:** 9.1 Credible source 5.6 Information about emotional consequences **Environmental restructuring** 12.1 Restructuring the physical environment 12.2 Restructuring the social environment 7.5 Remove aversive stimulus	Promote Responsive Feeding Practices Promote caregiver recognition of hunger & satiety cues Prevent overfeeding	Less Rapid Infant Weight Gain Improved Infant Appetite Regulation Promote Responsive Parental Feeding Habits and Behaviours

Motivation	Facilitator: caregiver emotions	Promote caregiver self-efficacy	16. parent caring for themself to care for their baby	Persuasion: 11.2 Reduce negative emotions 15.4 Self-talk 5.6 Information about emotional consequences 13.1 Identification of self as role model	Reduce Misperceptions around Infant Feeding Reduce Parental Internalized Stigma and Negative Emotions Towards Formula Feeding Promote Caregiver Self-Efficacy and Autonomy	Promote Responsive Parental Feeding Habits and Behaviours Promote Caregiver Wellbeing

6.4.3 Phase 3: Optimisation

6.4.3.1 Descriptive Statistics of BRIGHTs In the Wild Study Participants

22 participants responded to the recruitment post, of whom 21 were eligible and re-contacted via email. In total, 9 participants consented to take part, were provided access to the prototype, and asked to schedule a 60-minute follow up interview. Participants were offered a £50 in Love2Shop vouchers as a token of gratitude. Seven participants took part in the follow-up interviews, described below in **Table 6.5.** Caregivers were all mothers (100%) and were all in either a long-term relationship or married (100%). Overall, 58% of the sample was white, with 42% of the sample Asian, and all caregivers had achieved postgraduate level qualifications.

Table 6.5. Demographics of BRIGHT In the Wild Interview Sample (n=7)

Infant Feeding Exposures		*N*	Mean or %
Age of Participant at Recruitment (in years)		7	34.4
Age of Child at Recruitment (in weeks)		7	9.9
Parental Relationship			
	Mother	7	100%
	Father	0	0%
Relationship Status			
	Long Term Relationship or Married	7	100%
	Single	0	
Ethnicity			
	White	4	58%
	Asian / Asian-British	3	42%
	Black/African/Caribbean/Black British		
	Arab	0	0%
	Mixed or Multiple Ethnicities	0	0%
Education Level			
	Postgraduate Qualification	7	100%
	Undergraduate Qualification	0	0%
	A or AS Level	0	0%
	Vocational Qualification	0	0%
Infant Feeding Method in Milk Feeding Period			
	Primarily Formula Feeding	7	100%
Number of Children			
	One	6	86%
	Two	1	14%

6.4.3.2 Qualitative Feedback from In the Wild Interviews

Overall, all seven participants reported interacting with the prototype to prepare for the interview and for their own purposes within their day-to-day lives. Participants reported returning to information they found particularly useful, and most participants (6/7) expressed positive experiences when reviewing the resources. Caregivers felt the BRIGHT content provided evidence-based and unbiased formula feeding guidance that made them feel "validated" and "reassured". A few minor modifications and amendments in line with the APEASE criteria of intervention suitability were suggested and are reported below.

6.4.3.2.1 APEASE - Acceptability

Overall, participants reported BRIGHT's tone and language as highly acceptable, using descriptions such as - "friendly", "nice", "neutral", "factual", and "sensitive". The written materials were not seen as "lecturing" or "patronising", and therefore provided guidance without a dogmatic tone and took into consideration both the participants' home and family circumstances, as well as the individual characteristics of their child. Crucially, the language was deemed as easy to read and was "repetitive in the right places" (Participant 03). Parents also reported that none of the BRIGHT guidance conflicted with their parenting values, and where information was not in line with their personal parental preferences – such as BRIGHTS written guidance to help a baby fall asleep on their own – parents reported that these resources might have value for other parents and therefore they did not hinder their acceptance of the BRIGHT prototype. Parents found the app-based format of BRIGHT highly acceptable and feasible to use as an app "is perfect because honestly since the baby I open my laptop less" (Participant 04) and "you can [search for information] more easily one-handed" (Participant 06). Moreover, BRIGHT was seen as highly credible, which contributed strongly and was important to parents.

6.4.3.2.2 APEASE - Practicability

Participants found the resources easy to understand, providing simple and clear guidance, and free from unnecessary jargon. For example, one mother said BRIGHT was, *"as good as sort of speaking to… your health visitor or something"* (Participant 02).

6.4.3.2.3 APEASE - Effectiveness

To evaluate BRIGHT's effectiveness, we ascertained how BRIGHT might help participants engage in the key target behaviours identified as part of the Theory of Change model. Caregivers reported that BRIGHT made them feel confident when *selecting an appropriate formula* milk before or in the few days following birth. Parents reported common confusions and anxiety when making these decisions.

Regarding '*preparing an appropriate volume of formula milk'*, participants reported more varied levels of effectiveness. On one hand, participants felt that the BRIGHT guidance would reassure them when preparing a feed and they would refer to the guidelines moving forwards. However, it was also reported that the difference between the amounts of formula milk recommended on their personal formula milk tins and packages and the WHO guidelines provided within BRIGHT led to confusion. They indicated that they would, by default, refer to the amount indicated on the formula milk product "… because it's always there in front [of me]" (Participant 07), which was contrary to BRIGHT advice to utilise BRIGHT formula volume guidelines instead of packaging. However, after receiving the BRIGHT WHO formula milk volume guidelines, one parent voiced concern that she was offering her baby too much milk. This indicates that provision of the WHO guidelines remains useful in providing feedback on the key target behaviour and might perhaps reduce overfeeding. Finally, of note, one parent of a premature baby felt that the formula feeding guidelines were neither applicable to, nor representative of, their child.

For '*preparing a feed'*, the majority of parents already felt confident in preparing a feeding, however for a few participants (3/7) the content was reported to improve their bottle preparation behaviours. For example, one participant reported that they would level the scoop of formula powder, to avoid feeding too high a volume of formula milk. Whilst another reported that they would leave the boiling water to cool down for longer, as they were boiling the water "and then making it up straight away" (Participant 03), as to not lower the nutrients provided by the milk. It was also noted that some participants were using a formula preparation machine. As the NHS advises against using such machines, we did not include instructions on how to utilise these machines safely.[242] Whilst this absence was noted by some participants using these machines, they still felt the guidance on manual bottle-preparation was useful were they to prepare a bottle of milk manually.

All participants felt positively about the responsive bottle-feeding resources provided. Overall, parents generally felt the focus on responsive bottle-feeding provided them with new information and strategies that helped them to adopt more responsive feeding practices. This encouraged them to be more attentive to their infant's hunger and satiety cues. Specifically, the written content 'offer love before food' was well received and led to self-reported behaviour change in three parents, who reported trying this strategy. For example, one mother reported that "we actually [tried offering love before food] this morning… he started crying… so we just brought him downstairs and played with him… he actually went back to sleep after that" (Participant 03). Another mother reported that "I've kind of been overfeeding" (Participant 02) as she had been waking her baby regularly to feed, and therefore BRIGHT led to her making more effort to limit this behaviour. Moreover, one mother reported that BRIGHT helped her reframe a belief that overfeeding is not possible in early infancy, stating that "I'd always been told as well that you can't overfeed a baby, I'd never been looking for the cues which you're [BRIGHT] obsessed with… But now I am thinking about it more, like they're telling you what they need when they need it" (Participant 02). Finally, one parent reported that BRIGHT helped them better differentiate hunger from tiredness cues through the sleep article 'How can I tell if my baby is tired' and potentially reducing overfeeding practices when feeding in response to sleep, as opposed to hunger cues. Participants also reported that BRIGHT helped them set a better sleep routine, as well as helped them follow the suggested weighing schedule – reducing confusion and anxiety around how often to weigh their baby.

6.4.3.2.4 APEASE - Affordability

Overall, parents responded positively to the affordability of accessing BRIGHTs resources which would be free to download for all caregivers. Participants also reported that the BRIGHT articles could help families save money by advising them to avoid unnecessarily expensive formulas, given the common misperception that more expensive formula milks are of higher quality. Participants also reported that being given access to BRIGHT would save them time when seeking information, for example one mother stated *“having this information in Baby Buddy would have saved me probably a solid week’s worth of research that I did on my own”* (Participant 03).

6.4.3.2.5 APEASE - Side Effects and Safety

Participants observed and reported few minor safety implications and side effects. Crucially, one participant expressed concern that having access to the BRIGHT materials on different types of milk may have deterred her from trying and benefiting from anti-reflux milk. She suggested softening this messaging: “I think it's, it's best to mention… at the end… ‘you know at the end of the day, try it- if it works, it works…” (Participant 02). Other minor concerns included settling babies to sleep without using a feed, using a UV sterilizer as it is not covered in the BRIGHT resources, and guidance on throwing away unused formula milk. This feedback was integrated.

6.4.3.2.6 APEASE - Equity

Overall participants felt the BRIGHT materials were supportive, representative, and inclusive for parents included in our sample. For instance, one mother noted that BRIGHT was “really sensitive to everybody… sensitive to different cultures and backgrounds” (Participant 07). Moreover, parents reported that they felt BRIGHT represented their unique needs – with one mother stating that she felt BRIGHT was sensitive to her challenges and needs as a mother with obsessive-compulsive disorder. However, one participant expressed the desire for BRIGHT to offer more specific information for caregivers of babies born prematurely, stating that “I think it does [make you feel a little bit excluded]. It’s more that you always kind of feel like no one mentions it and I don’t know where I fit” (Participant 07).

6.4.3.2.7 Additional Modifications and Feedback

Some parents made wider requests for features that are intended to be implemented into BRIGHT – such as a search function, a formula feeding tracker, and growth centile feedback when using the growth curves in Baby Buddy. These modifications are outlined in Appendix XVIII.

6.4.3.3 Summary of Overarching Themes from BRIGHT prototype development

As a final stage of research, the research team looked across the PPIE and 'In the Wild' interviews to highlight relevant and novel themes regarding caregiver experiences. Five themes summarise the key perspectives of caregivers, which guided the development and modifications to the BRIGHT resources. These themes, alongside key descriptions, are summarised below in **Figure 6.11**. However, a full description of each theme, and how it was raised by participants, is presented below in the section 6.5.2.

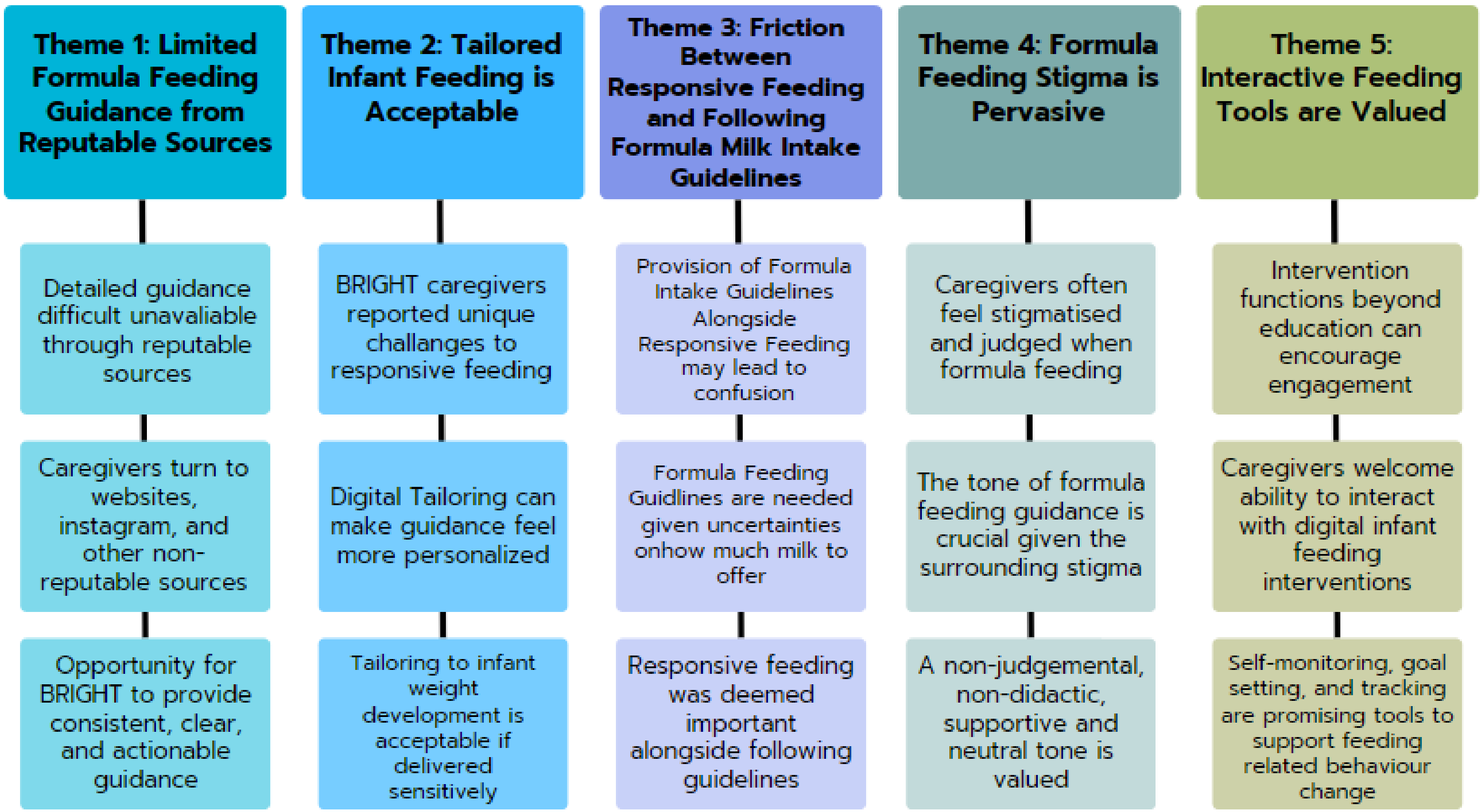


Figure 6.11. Overarching Themes and Subthemes from BRIGHT Prototype Development obtained from PPIE work and qualitative interviews with caregivers

6.5 Discussion

6.5.1 Summary of Findings

The present chapter outlines the development of a digital prototype for BRIGHT– The Baby Responsive Intervention for Growth & Health Tracking. Throughout my PhD I led on the development of BRIGHT's resources on formula feeding and related behaviours. BRIGHT was developed in two iterative stages in line with the PBA.[209] This iterative approach to development helped ensure that the resulting prototype was both acceptable and engaging to target users, but also feasible for families across the UK to implement. In the first stage we established the collaboration with Best Beginnings our charity partner, undertook a review of previous qualitative research to exploring the barriers and facilitators to caregivers following formula feeding guidelines and responsive milk-feeding behaviours, and performed a behaviour change mapping exercise of the Baby Milk Study.[24] Then, we recruited a diverse PPIE panel of caregivers (n=15) to amend the Baby Milk study resources and co-create the BRIGHT resources using our target population. This resulted in the co-creation of 39 written articles, 6 videos, 1 developed interactive weight feedback feature, and 2 written proposals for a formula feeding tracker and appetite quiz. During this development process, a theory of change model, applying the BCW and COM-B Model[202], was developed to illustrate how key barriers and facilitators to responsive and appropriate formula feeding behaviours would be addressed. In the second stage, we applied qualitative research or an In the Wild Study to optimise the BRIGHT prototype in line with the APEASE[222] framework for intervention evaluation. The BRIGHT prototype was deemed as highly acceptable, novel, and feasible to implement by caregivers. Nonetheless, numerous considerations and sensitivities need be considered in further stages of BRIGHT development and by future research on interventions targeting the infancy period. Within this discussion, I will reflect on these key considerations that emerged from the BRIGHT prototype development, in line with previous literature. Moreover, I will highlight my learning and reflections regarding potential opportunities and challenges for future digital interventions targeting the infancy period.

6.5.2 Discussion of Emergent Themes Compared to Previous Literature

6.5.2.1 Theme One; Scarce Formula Feeding Guidance from Reputable Sources

The first theme that arose was the limited reputable guidance and support currently available to support formula feeding caregivers. This finding mirrored a barrier to following formula feeding guidance highlighted within our rapid review of barriers and facilitators to following formula feeding guidance as well as a barrier to responsive infant feeding represented in the largest systematic review of barriers and facilitators to responsive feeding to date.[182] Moreover, similar to previous qualitative studies, caregivers commented on a reluctance from healthcare professionals to offer formula feeding guidance[169,172,174] in the early weeks of life, likely resulting from a fear of interfering with breastfeeding efforts or establishment.[174,235] In a previous survey of 270 Australian mothers, 20% of formula feeding mothers reported receiving no feeding advice from professional sources, versus only 5% of breastfeeding mothers who did not receive breastfeeding advce.[175] Moreover, findings from caregivers in the UK have reported similar frustrations and an inability to find adequate information on formula feeding best practices from reputable sources. [171,173,243] In particular, caregivers in the Baby Milk trial reported that these experiences with healthcare often led them towards negative emotions and feelings of guilt and perceived stigma[171]. I postulate that these negative experiences and emotions, may thereafter lead parents to refrain or hesitate when seeking further feeding support from the healthcare system. This possibility was also discussed by the BRIGHT PPIE panel. Hence, they emphasized that BRIGHT guidance should be communicated in a non-judgemental tone to avoid exacerbating existing feelings of guilt or stigma surrounding infant feeding.

BRIGHTs caregivers also emphasized that they wished that they had received formula feeding support, or to have been signposted to it as soon as they adopted formula feeding. This aligned with a key theme which arose from Baby Milk – where mothers commonly expressed wishing they would have had access to Baby Milk sooner, prior to the establishment of formula feeding habits that would later be more challenging to modify.[174] Taken together, these findings suggest that the early provision of reputable responsive bottle-feeding guidance could i) help to establish more responsive and appropriate formula feeding behaviours and habits during their period of establishment and ii) help to *buffer* negative emotions and stigma surrounding formula feeding. Moreover, parents in BRIGHT expressed that they had little choice but to turn to multiple, and often less reputable sources of feeding guidance. Hence, one mother involved in the In the Wild interviews expressed that "*having this information in Baby Buddy [the complete BRIGHT prototype] would have saved me probably a solid week's worth of research that I did on my own*". This quote demonstrates the onerous task placed on formula feeding parents to seek resources independently and from less reputable sources, such as formula milk company websites and social media. A recent systematic review evaluating the quality of feeding guidance provided in publicly available infant feeding apps also demonstrate that these resources can contradict public health guidelines.[177,244] Hence, there is an enormous and important opportunity to provide caregivers who use formula milk to feed their infants with accessible and reputable formula feeding guidance. I postulate that this provision carries the potential to support appropriate bottle-feeding behaviours, perhaps support infant weight development, as well as reduce negative emotions and guilt surrounding formula feeding. However, these hypotheses require testing in future stages of BRIGHT research.

6.5.2.2 Theme Two: Tailored Resources

An overarching aim of BRIGHT was to explore the acceptability and feasibility of offering feeding resources that are *tailored* to an infant characteristics (e.g. infant growth). Whilst, tailoring is a somewhat an inherent feature of face-to-face interventions, such as in Baby Milk where a facilitator could offer individualized advice to parents, tailoring in digital settings requires algorithms that match guidance to the users characteristics.[245] Whilst this digital tailoring has shown be an effective and promising method to promote behaviour change in digital health interventions related to physical activity and smoking[246,247] it's acceptability and feasibility for interventions targeting infant feeding has not been explored. Nonetheless, face-to-face tailoring offered in Baby Milk was demonstrated to buffer the relationship between higher infant appetite and higher formula milk intake[184]. This suggests that guidance that considers the individual challenges that parents face with their infant's appetite might be more effective at reducing overfeeding. However, the acceptability and practicality of such tailored components within digital infant feeding interventions is largely unexplored.

Looking to the insights from the BRIGHT's PPIE co-creation process, it was clear that BRIGHT caregivers reported highly unique barriers to appropriate and responsive milk feeding behaviours based on the individual appetite, temperament, and growth patterns of their child. Therefore, their ability to adhere to broad guidelines surrounding infant feeding, such as not pressuring a child to finish a bottle, depended on these unique characteristics of their child. As hypothesised, BRIGHT's PPIE panel and research participants found feeding guidance tailored to their infant's growth patterns highly novel, useful, and acceptable. Furthermore, receiving 'top feeding tips' specific to their infant's growth centile was seen as helpful and easy to implement, if delivered in a non-alarmist and supportive tone. Moreover, more generally, caregivers appreciated BRIGHT's acknowledgement that 'every baby is unique' and felt that BRIGHT recognition of individual differences across infants improved the acceptability and salience of the prototype.

Whilst no studies, to our knowledge, have explicitly explored caregiver perceptions of tailored infant feeding resources, when looking at the literature more broadly caregivers have reported a wish for more individualised feeding support. For instance, a recent review of maternal perceptions of the UK's Baby Friendly Hospital initiative highlighted a preference for feeding guidance that was tailored to them and their infants across both breastfeeding and formula feeding mothers.[173] However, these findings might once again reflect formula-feeding mothers feeling underserved by the healthcare system geared towards offering breastfeeding support. Nonetheless, a flexible approach to feeding support, that considered how 'no two babies are the same' was reported as far more engaging for mothers in this study. Whilst qualitative reports from caregivers demonstrate a desire for tailored support, it is important for future studies to test the implications of such support in practice. This is particularly relevant for weight-tailored guidance, as previous research with caregivers of infants has demonstrated that weight-centric guidance can be off putting.[172,182] Whilst within BRIGHT, caregivers did not feel that weight-centric and tailored messages were off putting, they emphasised that weight-related messaging needs to be delivered through a non-dogmatic, non-alarmist, and supportive tone. However, further research is needed to explore the influence of such feeding guidance tailoring on feeding behaviours as well as caregiver emotions and wellbeing.

6.5.2.3 Theme Three: Friction Between Responsive Feeding and Formula Milk Guidelines

Throughout the prototype development process, one emergent theme was parents perceived friction between following responsive feeding guidance in BRIGHT (e.g. a child led approach to feeding) and following the provided formula milk intake guidelines (e.g. a caregiver led approach to feeding). This friction has been recognised by previous studies. For instance, formula packaging and guidelines was highlighted as a physical cue that may contradicts a responsive feeding style in Redsell and colleagues review.[172,235] This friction was also reported in qualitative work with mothers participating in Baby Milk (n=19), as some mothers felt confused by the conflicting advice to both “stick to the tin [instructions],” whilst feeding on demand when their baby was demonstrating hunger cues.[171] Hence, we sought to utilise co-creation with parents to explore this friction further and reach an appropriate strategy for feeding guidance alongside the formula milk intake guidelines.

First, we deemed it important to offer parents formula milk intake guidelines given the high prevalence of feeding above the guidelines in Baby Milk[163] and parents reported uncertainties on how much milk to offer their child[171.] However, ultimately BRIGHT encouraged responsive feeding such that they did not pressure or restrict their infant if they did not meet the recommended amounts. Whilst only few studies have explicitly explored the influence of responsive feeding guidance on infant growth outcomes, one qualitative study of Australian mothers reported that without responsive feeding guidance caregivers increased the amount of formula offered and used pressure to ensure their infant consumed the recommended amount. [172] Moreover, two previous interventions which targeted responsive feeding behaviours - Baby Milk and NOURISH - both demonstrated more responsive feeding behaviours in the first year of life[186] and reduced rates of milk intake.[163] Moreover, a follow up analysis of the INSIGHT trial showed the intervention group demonstrated lower rates of emotional overeating in later childhood. This effect was also explained, or mediated, by the lower usage of feeding to soothe infants during the intervention.[248] Hence, we postulate that allowing responsive feeding to take priority, over strictly following the WHO guidelines, might result in both short-term and long-term promotion of responsive feeding.

Similar to previous studies, when taking this approach, some BRIGHT parents reported feeling confused when attempting to both feed in line with the guidelines and take a truly responsive or child-led approach to feeding. Hence, we found it useful to communicate to caregivers that the recommended daily intakes of formula milk provided by the WHO guidelines are not the recommended amounts for *every* baby, as they are based on estimated average requirements according to age and sex.[249] Therefore, the guidelines provide a helpful 'starting point' to estimate the appropriate amount of milk to offer their child. However, ultimately BRIGHT encouraged responsive feeding such that they did not pressure or restrict their infant if they did not meet the recommended amounts. Given the complexity and nuances of these recommendations we used co-creation with caregivers to reach the most acceptable and clear communication strategy. Overall, this was deemed sensible to caregivers if communicated clearly and relieved some underlying confusion on this topic.

However, parents also expressed the need for BRIGHT to recognise that this guidance may be more challenging for parents of a child with a higher or lower appetite. For instance, following a truly child-led approach to feeding may be a challenge with an infant of a lower appetite where parents are concerned for adequate milk intake. No studies to date have investigated the influence of this tension on measured feeding behaviours, or in the context of infant appetite. It is therefore important for future research to investigate the implications of responsive feeding guidance for children of varying appetites or growth patterns.

6.5.2.4 Theme Four: Formula Feeding Stigma is Pervasive

A key theme which emerged throughout the development of BRIGHT is the pervasive stigma attached to formula feeding. Caregivers involved in the development of BRIGHT felt that this stigma was rooted in negative experiences with healthcare professionals, cultural narratives around formula feeding, and the lack of support and guidance available to formula feeding families when compared to resources available to breastfeeding families. These negative emotions may act as a key barrier to seeking further support from the healthcare systems or adopting responsive feeding guidance. These insights confer numerous previous qualitative reports from caregivers – highlighting the inadequacy of support for formula feeding which lead to emotions of guilt, concern and even a sense of 'failure' when introducing formula milk.[171,173,175,243] Hence, we postulate that provision of reputable and comprehensive formula feeding guidance may help reduce negative emotions surrounding formula feeding and as we as internalized stigma, however this hypothesis needs testing under future research.

In Redsell and colleagues review of barriers and enablers to responsive feeding practices, parental emotions such as distress were identified a key barriers to responsive feeding.[167] Hence, considering parental wellbeing and emotions within feeding guidance may be an important element to promoting more responsive feeding practices. In line with this finding, BRIGHTs caregivers emphasized the importance of a supportive and non-didactic tone within infant feeding messaging. Whilst this was anticipated, given the sensitivity of infant feeding and care, parents also felt that if messages were unintentional delivered with any tone of judgement, BRIGHTs guidance can potentially exacerbate stigma that may already be affecting caregivers. As highlighted by previous qualitative studies[173] – caregivers are likely to enter formula feeding with numerous emotions given the cultural narratives around formula feeding, which can influence their perceptions of resources such as BRIGHT. Therefore, caregivers felt it crucial that BRIGHT as well as other infant feeding guidance or intervention be presented in a non-judgmental, supportive, and neutral tone. To achieve this in BRIGHT, it was crucial to undertake participatory research or co-creation using our PPIE panel, where caregivers could highlight messages which may be off putting or exacerbate any commonplace stigmas. I therefore urge future infant feeding interventions to make use of participatory approaches to ensure the acceptability of the intervention for the target population.

Finally, a novel insight which emerged from BRIGHTs development was caregivers' acceptance of guidance that could contradict their personal parenting philosophies, if the guidance was presented in a non-didactic manner. It is a common concern of public health interventions that evidence-based guidance might be dismissed or hinder the persuasiveness of the overall intervention where it contradicts the personal views of the participants. This concern may be particularly pertinent to the topic of infant feeding – which is highly emotive and many different 'schools of thought' exist. However, it was of interest many caregivers in the BRIGHT PPIE panel and In the Wild study pinpointed information that contradicted their personal views but felt it important to still include as a strategy for parents who might benefit from it. One example was BRIGHTs encouragement to refrain from using milk-feeding to encourage sleep, instead offered strategies to help an infant soothe themselves to sleep. Some parents felt this guidance contradicted their personal philosophies– and would prefer not to use such self-soothing strategies. However, as BRIGHT introduced these approaches with caveat that the decision to adopt this guidance was ultimately up to them as caregivers, they felt they were still acceptable to present. However, it is important to note that this approach may not be appropriate for crucial safety-related information, such guidance regarding co-sleeping. Moreover, this 'non-dogmatic' approach needed to be evaluated against caregiver feedback to ensure key messages and components of BRIGHT – such as weight monitoring – was also not lost through the *softening* of language. Once again, highlighting the value of co-creation with the in the development of digital health interventions.

6.5.2.5 Theme Five: Interactive Feeding Tools are Valued

A final theme which emerged from the development of BRIGHT, was caregivers desire for interactive tools to be integrated into BRIGHT. Whilst caregivers valued the written and video-based formula feeding guidance, as this was highly novel to them, they also expressed a desire for digital tools to monitor, track, and set goals around infant feeding. Moreover, caregivers reported having previously used and numerous mobile applications that allowed them to monitor, track, and set goals related to infant feeding. Therefore, integrating these tools into BRIGHT could help offer a 'complete formula-feeding package'. Moreover, behavioural science research demonstrates that interventions integrating such tools which allow for BCTs such as goal setting, self-monitoring, and feedback on behaviour are more effective at achieving their intended behaviour change.[222,223] As such, our Theory of Change model informed highlighted numerous BCTs that may indeed be effective in overcoming barriers to responsive feeding but would require the development of such interactive tools. For example, a formula feeding tracker would allow parents to self-monitor their formula milk provision, set goals to achieve the any desired reduction of formula milk provision, as well as provide parents feedback on their feeding behaviour. However, in the early stages of BRIGHTs development it became apparent that the financial costs of developing a formula feeding tracker was too high for the current phase of research. Hence, we decided to prioritise interactive features which would be affordable and central to the guiding principles of BRIGHT – which included the tailored weight feedback messages. Therefore, the development of two more expensive features – the formula feeding tracker and the appetite quizzes will be co-created and developed under in future stages of BRIGHT.

Finally, whilst parents expressed a strong desire for a formula feeding tracker to be integrated into BRIGHT, no studies to date have considered how these commonplace trackers might influence feeding behaviours for formula feeding caregivers. It has been proposed that feeding trackers, might in reality deter from responsive feeding and encourage more pressuring or restrictive practices if an infants is not tracking onto average estimates for their formula milk intake.[182] Hence, we urge future research to investigate the interaction between self-monitoring tools such as feeding trackers and responsive feeding behaviour in real-world settings.

6.5.3 Reflection on Opportunities and Challenges for Digital Interventions for Infant Feeding

6.5.3.1 Opportunity #1: Potential for Tailored Resources

Throughout the development of the BRIGHT prototype, several key opportunities and challenges for BRIGHT alongside other digital interventions targeting the infancy period became apparent. One opportunity that became increasingly apparent was the potential for digital interventions to offer feeding guidance that is tailored to individual characteristics of infants – such as their growth or appetite. Whilst face to face interventions can inherently offer tailored guidance – such as within the Baby Milk, NOURISH and INSIGHT trial[56,163,185], this personalisation can be challenging for digital health interventions to mirror. However, recent developments in AI do allow for the delivery of information to be personalized to an individual user and their characteristics. Yet, the acceptability and uptake of such tools is largely unexplored. This is a particularly important and promising avenue for future research as there is a literature demonstrating the utility of goal setting and self-monitoring as useful BCTs to change dietary behaviours.[222,223,250] Hence, offering parents digital tools to enact intervention functions beyond education and persuasion, may be a more effective approach to change infant feeding behaviours. As aforementioned, caregivers from the BRIGHT PPIE panel responded positively to tailored resources and interactive tools, and some parents voiced the use of such tools – such as feeding trackers or timers – on other apps or websites. However, whilst these digital tools may be theoretically promising for behaviour change and already commonly used through publicly available apps, no studies have explored the implications of 'feeding trackers' on feeding behaviours. As aforementioned, encouraging parents to use feeding goals and plans to reduce overfeeding might have unintended consequences in encouraging a more controlling and less responsive approach to feeding. Hence, there is a significant opportunity for future studies to test the application of tailored infant feeding tools– such as feeding trackers.

6.5.3.2 Opportunity #2: Generate More Rigorous Evidence for Infant Feeding Practices in Relation to Infant Growth

A second opportunity of BRIGHT lies in the lack of rigorous or experimental evidence measuring the relationship between responsive feeding and infant weight development.[19,251–253] Whilst responsive feeding is widely recommended by reputable sources and the NHS, there is dearth of evidence demonstrating robust associations, apart from observational studies which are subject to confounding from both social and infant related factors. Moreover, given the presence of reciprocity towards infant weight patterns measured under Study 2 of this thesis and previous studies[40,70], it may be easier to practice responsive feeding towards an infant who is growing at a steadier pace – resulting in a measured association between responsive feeding and more advantageous weight patterning. In this scenario – it is difficult to ascertain cause and effect in this relationship and establish whether responsive feeding truly caused healthy growth patterns. Hence, there is an urgent need for higher-quality experimental designs, such an RCTs, to establish cause and effect in the relationship between responsive milk-feeding and infant weight outcomes. Whilst no studies have examined responsive milk-feeding, findings from the BLISS RCT (n=206) demonstrate that a responsive or 'baby-led' approach to complementary feeding, when compared to a more traditional 'spoon-fed' approach, did not lead to lower BMI z-score at 12-months or 24 months.[254] However, the BLISS 'baby-led' approach group showed lower satiety responsiveness and food fussiness at 12-months, suggesting that a responsive approach to complementary feeding might carry benefits for children's appetite regulation.[254] However, once again, further evidence is needed to support the case for responsive feeding in milk-feeding, particularly given their ubiquity and emphasis in the current feeding guidelines. We therefore see an enormous opportunity for BRIGHT, when taken forward to a RCT or quasi-experimental trial design, to help explore the influence of responsive milk-feeding behaviours on infant growth under experimental settings. Moreover, it would be of value to explore whether responsive feeding guidance may lead to varying outcomes for children with different appetite profiles. For example, could responsive feeding guidance (e.g. advice feed on demand in response hunger cues) lead to overfeeding amongst infants with a more responsive and demanding appetite? Exploring these complex interactions between responsive feeding, appetite, and growth under experimental conditions carries enormous potential to shape responsive feeding guidance that addresses complex parent-child reciprocity in infant feeding.

6.5.3.3 Challenge #1: Conflicting Timelines Between Research and Third Sector Organisations

The first and most pertinent challenge that arose in BRIGHT was the conflicting timelines between fast-moving charities, such as our partner Best Beginnings, and the lengthy timelines of evidence gathering. For example, to undertake a future RCT with BRIGHT, would take years of planning, piloting, execution and data analysis. As these stages would have to be enacted prior to the 'roll-out' of BRIGHT on the Baby Buddy app, to remove the risk of cross-contamination between a control and intervention group as well as any unintended harms resulting from the intervention, it would be numerous years before the public would come into contact with BRIGHT. This is a common challenge that halts he speed at which novel public health interventions can be delivered to the public. However, for our charity partners Best Beginnings a significant priority remains to meet the needs of their users as fast as possible when these needs are identified. As their users were increasingly expressing the need for more formula feeding resources, which BRIGHT was developing, they felt an ethical challenge to deliver this support prior to the completion of a future RCT trial. At present, we are exploring alternative trial designs, such as either i) pragmatic trials where an intervention could be released to the public once participants are randomised to their intervention group, or ii) a more real-world approach to intervention evaluation which would gather less rigorous evidence but more rapidly. Nonetheless, whilst partnerships between charities research teams may be incredibly fruitful – this conflict is a common challenge worth bearing in mind for future interventions.

6.5.3.4 Challenge #2: Funding for Digital Health Interventions

A second key challenge is accessing appropriate and sufficient funds for digital health interventions. Digital interventions require significant funds for not only technical development costs, but also the upkeep and dissemination of technical features to support users ongoing engagement. However, few funds available through public health research funding bodies allow for or allocate significant funds towards such development costs. Instead, they largely allocate funds towards evidence gathering and research costs. Hence, accessing sources of funding that provide adequate support for the technical development of interventions such as BRIGHT, alongside evidence gathering, remains a challenge for our team and other digital health researchers. Moreover, whilst interactive digital tools that integrate BCTs such as self-monitoring, goal setting, and feedback may be more effective in changing behaviour and anticipated by intervention users - these tools are expensive to develop and maintain. Hence, within the initial planning phase, it became clear that BRIGHT would not have the funds to develop as many interactive features (e.g. formula feeding tracker) as initially planned. Hence, once again, we urge intervention developers to consider the costs of digital development and accessing additional funds to support the development of features in early stages of intervention planning.

6.5.4 Merits and Limitations

6.5.4.1 Merits

Overall, the development of the BRIGHT prototype benefitted from its use of the PBA for intervention development. The PBA is a rigorous, systematic approach to designing a digital intervention to meet the psychosocial needs of its users.[209] Specifically, by integrating perspectives of target-users into each phase of development, the PBA helps to ensure that digital interventions are attractive, persuasive, and feasible to implement. Moreover, as formula feeding and infant growth is a subject surrounded by significant stigma and sensitivity[171,173,243], co-creation with parents to ensure the tone and messaging was appropriate is a significant merit to the developed BRIGHT prototype. It was of merit that BRIGHT involved a diverse set of target user caregivers in the PPIE panel – including both mothers and fathers to ensure varying perspectives were captured. Moreover, in line with the PBA, we implemented systematic intervention development frameworks such as a theory of change model, rapid tabulation of PPIE feedback, as well as the APEASE criteria to help mitigate researcher biases that might influence the development of BRIGHT. Finally, the BRIGHT prototype benefitted from its use of qualitative process evaluation, or our 'in the wild' study to measure not only the acceptability and feasibility of the prototype, but also caregivers' engagement with the prototype in the context of their day-to-day lives. Digital health interventions which have been shown to be highly acceptable, through qualitative research and feasibility studies, have also shown surprisingly low levels of user uptake and retention when applied to a real-life settings.[219] Moreover, long-term participant retention and engagement is one of the most pervasive challenges in implementing digital health interventions.[211,220] Therefore, the use of real-time engagement-testing approaches, that can anticipate barriers to sustained engagement in the 'real world' are strongly encouraged for the optimisation of novel digital interventions.[213]

Finally, an unanticipated but significant merit to the BRIGHT prototype which emerged from caregiver feedback, was BRIGHT potential to benefit both infant health, but also support caregivers' mental health. Caregivers felt the lack of supportive and informative guidance regarding formula feeding was often detrimental to caregiver wellbeing in the period of early infancy, which is demanding emotionally and physically. Hence, offering BRIGHT to caregivers through the free and easily accessible Baby Buddy app could help to reduce these negative emotions, stigma, and support mental health. Caregivers involved in BRIGHTs development unanimously reported the benefits they would have felt if they had been provided with BRIGHT earlier in their feeding journey. However, these impacts need to be tested in practice within future research.

A final key merit to BRIGHT is its novelty and potential scalability. Given Baby Buddy's substantial reach across the UK with downloads from over 350,000 parents with and an average of ~7,000 new users every month[207] - the BRIGHT intervention has substantial potential to support caregivers across the UK. Moreover, given Baby Buddy's significant representation and reach within families living under greater deprivation, the delivery of BRIGHT through the mobile app can help ensure its delivery to underserved families and communities to potentially buffer existing health inequalities.

6.5.4.2 Limitations

First, although we utilised the BCW to better target caregivers' capability, opportunity, and motivation towards formula feeding behaviours, we recognise that BRIGHT does not yet offer goal setting, action planning, and self-monitoring functions within the Baby Buddy app due to aforementioned high development costs. There is a literature base supporting the utility of goal setting and self-monitoring to enhance behaviour change – particularly in overcoming the intention-behaviour gaps for health behaviours.[211,222,250] Hence, we produced prototypes for these features to be developed and tested at a later stage of research. Secondly, while all efforts were undertaken to create a diverse PPIE panel and qualitative sample, the sample of parents who took part in the 'In the Wild' study consisted of only mothers of a high educational attainment. Hence, the extent to which results regarding the APEASE criteria can be generalised to caregivers living in deprivation, with lower education attainment, or partners and fathers needs to be further considered.

Finally, we did not evaluate the BRIGHT prototype with any same-sex families due to recruitment challenges. As same-sex families are more likely to formula feed and have distinct needs and face separate stigmas[255,256], further evaluation work amongst LGBTQIA+ families should be undertaken. As Best Beginnings is currently running a separate project on developing resources for LBGTQIA+ families, this existing pool of families could be used in future BRIGHT development and evaluation.

Finally, it is important to reflect on sampling biases in the BRIGHT PPIE panel and the research participants in the 'In the Wild' study. As we mostly used Baby Buddy to recruit, to mirror the characteristics of Baby Buddy users to which BRIGHT would eventually be delivered to, it is possible that we might overrepresent the perspectives more health-conscious and tech-literate parents that are more willing to participate in a digital health study. A wider limitation of the qualitative methods utilised under BRIGHT's development is also the influence of potential researcher bias. While interview procedures and the interpretation of participant feedback are both subject to inherent bias from the involvement of the researchers, we used semi-structured interview schedules and rigorous components of the PBA, such as rapid tabulation, to mitigate these potential biases.[209] Finally, it is worth reflecting on that BRIGHTs resources were primarily developed to be delivered to primary caregivers of infants. However, there may be many other important stakeholders in feeding and infant care behaviours – such as grandparents, carers, or other family members. Hence, future stages of BRIGHT development and implementation will seek to explore how we can address these stakeholders and their influence on feeding behaviours throughout the BRIGHT intervention.

6.5.5 Future Directions for BRIGHT

Following the current stage of prototype development outlined in the current chapter, a formal evaluation of the BRIGHT intervention is planned (e.g. in a RCT or 'real world' evaluation). The extent to which this trial will consist of a randomised design (with a control group) is currently being decided with Best Beginnings, our charity partner due to the aforementioned time which these designs require. Regardless, this evaluation will be undertaken prior to BRIGHT's general release on Baby Buddy to test the influence of formula feeding guidance on i) feeding behaviours ii) infant growth outcomes and iii) parental wellbeing measures. As well as test for any unintentional harmful consequences.

As a novel 'feature' within the existing Baby Buddy App, BRIGHT will ultimately be delivered, at no cost to the user or health care providers, and at a national level. BRIGHT therefore has the potential to reach thousands of caregivers across the UK – particularly those living under greater deprivation who are often underserved. Moreover, we will also seek funding to develop and test the interactive features which were not possible to develop under the current funds – including i) a formula feeding tracker and ii) appetite quizzes for milk-feeding and complementary feeding. Overall, we will continue our collaboration with Best Beginnings to offer caregivers across the UK the first evidence-based and reputable library of formula feeding guidance. Moreover, if possible, a more rigorous quasi-experimental or RCT will allow BRIGHT to gather important evidence regarding the influence of responsive feeding on infant growth and health outcomes.

6.5.6 Concluding Statement

In summary, this chapter presented the protocol for BRIGHT, a novel digital intervention targeting responsive bottle-feeding practices to promote healthy growth amongst formula fed infants. Throughout the development of BRIGHT, caregivers highlighted the dearth of support available to formula feeding families through the healthcare system and other reputable organisations in the UK. Caregivers also reported experiences of stigma and negative emotions surrounding formula feeding. Hence, we observed a significant opportunity to support formula feeding families with comprehensive, non-judgemental, and evidence-based feeding guidance. The BRIGHT caregivers also highlighted several opportunities for digital interventions to better support responsive and appropriate formula feeding behaviours. Specifically, parents found feeding guidance which was tailored to the unique characteristics of their child (e.g. their infant's unique growth) as highly acceptable. Hence, feeding guidance should consider the highly unique barriers that parents face when adopting feeding guidance, and refrain from taking a 'one size fits all' approach. Moreover, interactive digital tools (e.g. a formula feeding tracker) was seen as highly useful and promising in promoting behaviour change. However, the future evaluation of BRIGHT seeks to test the influence of such tools on infant feeding behaviours in practice and help to establish more rigorous experimental evidence regarding the influence of responsive infant feeding on infant weight outcomes. Whilst we believe interventions such as BRIGHT carry significant potential to support healthy growth in infancy as well as caregiver well-being, these effects need to be measured in future studies.

Chapter Seven. Concluding Discussion

7.1 Summary of Thesis Findings

The overarching aim of the current thesis was to explore bio-psycho-social interactions between IFMs and infant weight development across the first year of life, and then apply these insights to the development of BRIGHT – a novel infant feeding intervention.

Part one of this thesis (Studies 1-3) triangulated between-families bi-directional epidemiology with discordant and classical twin designs to examine parent-child reciprocity between IFMs and infant weight gain trajectories in the population-based Gemini Twin study. These studies examined both *parent to child* and *child to parent* directions of influence in the relationship between IFMs and infant weight development. First, looking at *parent to child* influences, Study 1 demonstrated steeper weight gain trajectories across the first year of life for infants fed either with formula milk or a combination of breastmilk and formula milk, as compared to exclusively breastfed (EBF) infants, according to a crude 3-group measure of IFM. Using a more detailed 7-group measure of IFM, I showed that infants fed predominantly breastmilk from a bottle showed steeper weight gain than breastfed infants fed directly from breast – implicating bottle-feeding as a potential mechanism in the promotion of rapid weight gain. Finally, leveraging the discordant twin design, in attempting to remove residual confounding from shared environmental exposures, I found that the weight gain of twin pairs discordant for IFMs did not differ significantly. However, twins fed with more expressed breastfeeding or formula feeding showed a trend towards being smaller than their co-twin in early infancy. This novel finding pointed towards parent-child reciprocity - suggesting that a twin who is gaining weight more slowly than his or her co-twin would be given more expressed breastmilk or formula milk to encourage them to gain weight more quickly. Study 2, therefore, explored the *child to parent* direction of influence, by combining bidirectional longitudinal epidemiological analyses with the discordant twin design to further explore parent-child reciprocity in infant feeding and infant weight gain. Slower weight gain in the first weeks of life and higher maternal concern for low weight gain were both associated with a higher likelihood of parents introducing formula milk. However, in the period following the introduction of formula milk, infants fed with formula milk gained weight more rapidly to 1-year of age when compared to infants who continued to be exclusively breastfed. This suggests formula milk may indeed be an effective strategy for promoting faster weight gain. Therefore, I conclude that both the parent to child and *child to parent* directions of influence are important to consider in the relationship between IFMs and infant weight development. Finally, Study 3 explored potential gene-environmental interplay between infant feeding and child genetic liability towards infant weight gain. Using the classical twin design, I tested for two different types of gene-environment interplay: 1) whether the introduction of formula milk is a response to an infant's genetic liability towards slower weight gain in early infancy (i.e. a GE correlation); and 2) whether exclusive breastfeeding has the potential to reduce expression of genetic liability towards rapid weight gain in the first year of life (i.e. a GE interaction). No evidence of any gene-environment interplay between IFMs and infant weight gain was shown in Gemini.

In the second part of the PhD (Study 4) I described the development of the BRIGHT prototype (Baby Responsive Intervention for Growth & Health Tracking), a novel digital intervention which aims to reduce RIWG among formula fed infants by supporting responsive bottle-feeding. BRIGHT integrated findings and insights from part one of this thesis, ensuring that parent-child reciprocity in infant feeding was central to the resource's development. Moreover, BRIGHT's development utilized the Person Based Approach (PBA) for intervention development, to ensure the resulting prototype addressed the psychosocial needs of its users as well as barriers and facilitators to appropriate and responsive bottle feeding. Overall, BRIGHT was deemed acceptable, novel, and engaging by target caregivers. Numerous insights were raised by these caregivers regarding the delivery of BRIGHT and other infant feeding interventions, which will be discussed in this chapter.

7.2 Discussion of Findings from Part One of Thesis

7.2.1 Implications for Theory, Policy, and Practice

7.2.1.1 Implicating Bottle-Feeding in Rapid Infant Weight Development

Study 1 sought to explore the prospective association between IFMs and infant weight gain trajectories – using both detailed measures of milk-feeding modalities and detailed weight gain trajectories. First, using a crude 3-group measure of IFM, I observed that both formula-fed infants and those fed with a mixture of formula and breastmilk showed steeper weight trajectories compared to EBF infants, supporting observational findings from numerous other birth cohorts using broad measures of IFMs[52,53]. This suggests that the observed risk of RIWG is not limited to infants fed exclusively with formula milk, and those fed with a combination of breast and formula milk may benefit from interventions seeking to modify infant feeding behaviours or and support healthy infant growth.

Second, I utilised a more detailed measure of IFMs to explore potential mechanisms which explain RIWG amongst formula milk infants. Whilst few studies have directly examined potential mechanisms promoting rapid growth in formula fed infants, many propose that rapid growth is attributable to the different nutritional makeup composition of formula versus breastmilk.[109] An alternative hypothesis has emerged whereby behavioural differences in feeding that occur when feeding through a bottle and not the breast might also explain RIWG.[107,145] As these two mechanisms (bottle feeding vs. milk content) remain largely unexplored, I compared weight gain trajectories between infants fed breastmilk through the breast versus through a bottle, who offer a unique opportunity to examine the potential influence of bottle-feeding without confounding from differences in nutritional composition. In line with one previous study of the American Infant Feeding Practices Study II, infants fed predominantly breastmilk through a bottle showed steeper increases in weight gain to one-year of age, as compared to exclusively breastfed infants fed from the breast.[50] Whilst our findings would benefit from replication in alternative samples with similarly detailed measures of IFMs, bottle-feeding and its associated behaviours may be an important mechanism underlying accelerated weight gain amongst infants fed with formula milk. However, it is important to recognise that infants fed expressed breastmilk included those exclusively fed this way as well as those who were *primarily* fed this way, and therefore were given some formula feeding. This was necessary, given the rarity of exclusive expressed breastfeeding to ~3-months of age (n=14 Gemini Infants). Hence, the small proportion of formula given to these infants may have influenced their weight gain. Nonetheless, this proportion of formula feeding was small, and previous findings in exclusively expressed fed infants confer these results.[50] Therefore it is reasonable to infer from these findings that bottle-feeding is one potential mechanism, alongside the nutritional differences of breast and formula milk, which could promote RIWG in the first year of life. Similarly, the small (n=39) and perhaps distinct group of infants primarily fed with expressed breastmilk to ~3-months of age, may have been fed in this manner due to health complications, which should be considered in interpreting their growth trajectories.

In light of these results, future studies should seek to explore *which* specific bottle-feeding mechanisms and behaviours might promote RIWG in order to highlight specific targets for interventions. Whilst no studies look at such interaction between IFMs and parental feeding behaviours in early infancy, studies from later toddlerhood suggest that more responsive feeding in toddlerhood, which is more common in breastfed infants, may partially mediate the benefits of breastfeeding on healthy weight gain.[257] Whilst these interactions need to be explored earlier in infancy, these novel findings might imply that RIWG prevention might not only be a achieved through breastfeeding promotion but also interventions seeking to promote appropriate bottle-feeding behaviours amongst infants receiving formula milk. As families introduce formula milk for numerous reasons, some of which are outside the control of the mother (e.g. illness, limited breastmilk supply, mastectomy or adoption)[152] this more inclusive approach to feeding support would carry significant benefits for the majority of families in the UK who use formula milk to feed their babies[142]. Furthermore, given the lack of guidance and bottle-feeding support from reputable sources available to formula feeding caregivers, there is an enormous opportunity for bottle-feeding guidance to promote healthier growth from the very start of life. Overall, these findings suggest that a greater focus on the *how* of milk feeding (e.g. PFPs and responsive feeding), as opposed to solely the *what* of milk feeding (e.g. formula feeding versus breastfeeding) is important for future research, interventions and policies to support healthy weight development from the start of life.

7.2.1.2 Social Confounding in the Relationship Between Infant Feeding and Growth

For the final aim of Chapter 1, I used the DT design to examine the influence of IFMs on weight gain trajectories, removing residual confounding from shared environmental exposures such as family socioeconomic position. This is an important potential challenge to address in infant feeding research, given the strong social patterning of both breastfeeding and infant and child weight development in the UK.[43,112] My results did not show significantly steeper weight gain trajectories in twins fed with more bottle feeding or formula feeding. These findings align with the few family designs applied to breastfeeding and infant or child weight outcomes - only 1 of 4 previous sibling studies identified a significant association between breastfeeding and infant or child weight (in this particular case, risk of overweight from age 2).[45] The benefits of breastfeeding on infant weight development that are observed in samples of unrelated individuals may reflect, at least partly, other socioeconomic advantages of infants whose mothers breastfeed them (e.g. healthier diets and lifestyles in later infancy and early childhood). However, whilst the DT or discordant sibling design removes confounding from shared environmental exposures, it does not remove confounding from non-shared exposures[35] – such as unique infant weight gain or illness – which can influence both IFM as well as infant growth outcomes. On the relatively rare occasion that twins are fed differently, it is likely to be a response to differences in other non-shared factors such as health problems or differences in weight development. This was apparent in the DT trajectories of infant weight gain, where co-twins fed with more bottle- or formula feeding appeared to be smaller in early infancy - which I postulate reflects the introduction of formula feeding in response to a twin with slower weight gain. In this way, the association between IFMs and infant growth may be less observable amongst twins who are fed differently, not only due to the removal of social confounding, but also due to important non-shared differences between the twins. This is important to consider, as my findings cannot conclusively determine that the lack of association between IFMs and infant growth under the DT was entirely the result of removing social confounding. Future studies in infant feeding research, which to date has largely relied on observational studies of unrelated individuals, need to incorporate and triangulate study designs which are better able to remove confounding from social exposures (e.g. using either quasi experimental or family designs such as the twin design) as well as remove confounding from non-shared characteristics (e.g. experimental designs) where possible.

7.2.1.3 Parent-Child Reciprocity in Infant Feeding; an Important Avenue for Research and Interventions

Leveraging the twin design with bi-directional epidemiological analyses, Studies 1 and 2 measured important and novel parent-child reciprocity in infant feeding. In Study 1, weight gain trajectories between twins fed discordantly in Gemini suggested that twins who were fed with more bottle feeding or formula feeding to ~3-months of age were smaller in early infancy. This somewhat unexpected finding suggests that the introduction of bottle or formula-feeding may be a parental *response* to slower growth in one of their twins. Hence, these findings began to paint a more complex picture of the relationship between IFMs and infant growth[153] – challenging the cause-and-effect relationship between feeding (the postulated cause) and infant growth (the postulated effect). Therefore, Study 2 leveraged bidirectional epidemiological analysis and the discordant twin design to explore this reciprocity towards infant weight gain in more detail. This study demonstrated that slower infant weight gain as well as maternal concern for low weight gain in the early weeks of infancy increased the likelihood of parents introducing or supplementing with formula milk. I also found that parents reported higher levels of concern for low weight gain for a twin who was fed with more bottle-feeding or formula feeding than their co-twin.

These findings confer those of two previous singleton samples [40,70] which have demonstrated reciprocity towards slower infant growth in infant feeding. Therefore, these findings contribute significantly to the burgeoning evidence base supporting the complex and dynamic interaction between caregiver and infant, which shapes infant feeding experiences. Nonetheless, as very few studies to date have considered the reported bidirectional influences in infant feeding, our current theoretical understanding of infant feeding is incomplete. I would therefore urge future study designs to better consider and account for parent-child reciprocity when measuring associations between feeding exposures and growth outcomes, both during after infancy.[148] Secondly, I would urge future research to explore alternative infant characteristics (e.g. appetite traits) which might also influence IFMs and practices, to gain a more complete picture of parent-child reciprocity in feeding behaviour. It would be particularly useful to explore how alternative aspects of infant feeding – such as parental feeding practices in milk-feeding or complementary feeding – might be shaped by infant characteristics (e.g. weight development or appetite). If reciprocity is present within these more behavioural aspects of feeding, then interventions seeking to modify these behaviours would benefit from considering the unique barriers that arise from infant characteristics when parents attempt to adopt best-practice feeding behaviours.

Regarding implications for policy and practice, the presence of parent-child reciprocity in infant feeding suggests that the current 'one-size fits all' approach to feeding policies and interventions is unhelpful. For example, offering support to address caregivers' concerns surrounding weight faltering or slow weight gain, when there is no sign of clinically relevant weight faltering and the family wishes to continue breastfeeding, may be a useful strategy to support longer breastfeeding duration. However, future research should seek to explore the correlation between parental reports of concern for low weight gain and the prevalence of clinically relevant weight faltering. If there is a strong correlation, encouraging parents to continue breastfeeding when they report concern about their child's weight gain, may lead to unintended and harmful consequences for their child's health. However, if parental concern for low weight gain is common in the absence of clinically relevant weight faltering, then offering support to reassure caregivers about their concerns without the supplementation of formula milk, may prove useful in supporting longer breastfeeding durations. In line with this latter hypothesis, researchers have speculated that parental concern surrounding low weight gain may be exacerbated by the UK-WHO growth standards used in the UK.[11] These growth standards represent the growth of infants who were EBF or predominantly breastfed during the first few months of life, and who met a strict set of socio-economic and health related criteria. However, these infants may have continued to be EBF during the first few months of life *because* they had faster early weight gain and because there were no other major weight or health concerns[41,112]. Concern may therefore be heightened by these somewhat inflated growth references leading parents to supplement with formula milk, or HCPs to advise parents to supplement breastfeeding with formula milk. However, it is important to note that the wishes of the family and caregivers remains critical to consider in these interactions. For instance, encouraging a caregiver to continue breastfeeding where they do not wish to and wish to be supported with formula feeding instead, may exacerbate negative emotions and stigma that surround formula feeding.

More broadly, my findings also suggest that 'one-size fits all' initiatives and policies that have been implemented to support breastfeeding (e.g. the Baby Friendly Initiative[139]) may be limited as they do not actually address important child-led barriers to breastfeeding. Hence, feeding interventions might not only be more effective but more engaging and motivating if they take into consideration dynamic interactions between mother and child that shape feeding experiences. This suggestion mirrors that of previous reports[153] such that feeding polices should acknowledge the unique tensions or challenges that arise in every mother-child feeding relationship because of early emerging infant characteristics (e.g. weight faltering or infant appetite) and not as 'a fault' of the mother. This more individual, flexible, and non-judgemental approach to feeding support might result in more advantageous feeding behaviours as well as reduce commonplace negative emotions and stigmas that surround infant feeding. Greater recognition of child-led drivers of formula milk supplementation might help to shift some of the 'blame' and stigma that parents feel when adopting formula feeding to support the growth of their child. Researchers, policy makers, and healthcare practitioners should recognise the important role of parent-child reciprocity to shape more rigorous research and successful feeding interventions in the future. Nonetheless, whilst my findings highlight early weight gain as one of the likely determinants of formula milk supplementation, it is important to continue to consider numerous other determinants of feeding decisions – such as wider social, economic, and cultural influences.[112] As any theorical understanding of infant feeding would be incomplete without consideration of *both* infant-led *and* socioeconomic-led determinants of feeding, both need to be considered. However, to date the spotlight has largely been on structural social, economic and cultural drivers of infant feeding.

7.2.1.4 Formula Milk Supplementation: An Effective Strategy to Promote Weight Gain

Whilst Study 2 highlighted the important influence of early infant weight gain on infant feeding decisions, it also demonstrated that, after its introduction, formula milk supplementation was associated with steeper increases in weight gain. Infants introduced to formula milk 'caught up' to their EBF counterparts approximately 8-weeks after formula milk was introduced and continued to gain weight more rapidly to 12-months of age. Hence, the introduction of formula milk might indeed be an effective strategy used by parents to encourage weight gain in the first months of life. The findings from this thesis indicate that both infant growth can influence IFMs, and IFMs can influence growth – in a dynamic interactional process. In line with this, future studies should explore the potential mechanisms which might explain how and why formula feeding encourages steeper weight gain following its introduction – as proposed earlier in this discussion.

Mainstream hypothesis largely focusses on the potential programming of growth that occurs as a result nutritional differences between formula milk and breastmilk.[109] However, bottle-feeding behaviours and practices might contribute to rapid growth – through overfeeding or reduced capacity for infant appetite regulation. This latter *behavioural* pathway might be particularly apparent for infants who experience weight faltering or slower weight gain from birth. I postulate that caregivers might be more likely to introduce formula milk using a more pressuring approach to feeding due to their prior concerns for their infant's slow weight gain. In this way, it might not only be *what* an infant is fed (i.e. formula milk) but *how* an infant is fed that contributes to rapid growth following the supplementation of formula milk. Moreover, these feeding practices may lay the groundwork for future parent-child feeding dynamics that underlie future child feeding. I propose that future research investigates alternative aspects of milk feeding – such as bottle-feeding practices – which might be responsive to infant weight development to inform targets for intervention. These interventions might be particularly beneficial to infants who have been supplemented with formula milk due to concerns about poor weight gain. Nonetheless, these results suggest that further support for feeding behaviours amongst infants fed with formula milk carry potential to reduce the risk of RIWG, although this hypothesis needs to be tested in both trial settings and observational designs.

7.2.1.5 GE Interplay in Infant Feeding and Weight Development

In Chapter Four, I presented the first investigation of GE interplay in the relationship between IFMs within the first ~3-months of life and infant weight development using the classical twin design. Within this study, I investigated the potential presence of both GE correlation (testing the hypothesis that parents introduce formula milk in *response* to their infant's genetic liability towards slow weight gain in early infancy) and GE interaction (testing the hypothesis that the introduction of formula milk enables a greater *expression* of genetic risk for RIWG across the first year of life). Overall, I found no evidence of either GE correlation or GE interaction in this sample.

Firstly, IFMs reported in the first 3-months of life did not appear to be responsive to genetic liability towards slower infant weight gain from 0-3 months of age (a GE correlation). Twin pair concordance for IFMs was very high between both MZ and DZ twin pairs in Gemini suggesting there was virtually no genetic contribution to variation in IFMs. One explanation for these results may be that reciprocity towards genetic liability for weight may not emerge in *early* infancy where heritability of weight is lower (38% at 3-months in Gemini) than in later infancy (62% at six months in Gemini).[52] Therefore IFMs used in early infancy may more largely driven by i) social and economic factors as indicated by the significant portion of variation in RIWG from 0-12 months explained by shared environmental influences (C) and ii) fluctuations in infant weight gain or perceptions of infant weight gain (as highlighted in studies 1 and 2) as opposed to the genetic liability over weight gain itself. However, the use of a broad 3-group IFM measure – necessary to increase statistical power for twin comparisons – might have limited the ability to capture smaller infant feeding discordance. Therefore, future studies using alternative measures of infant feeding that can capture more discordance in feeding (e.g. volume of formula milk offered or PFPs) might be useful to explore differing ways in which GE correlation may emerge within infant feeding practices. Taken together, my results do not support the hypothesis that parents adapt their IFMs in response to their child's genetic liability for slower weight gain in the early months of infancy. Reciprocity towards genetic influences over weight might emerge in later life as demonstrated by studies in childhood. However, as my study is the first to explore GE correlation in IFMS, our findings require replication in alternative samples.

Secondly, to explore GE interactions, I hypothesized that the heritability of RIWG at 12-months of age would be significantly higher amongst infants fed with formula milk, either exclusively or in combination with breastmilk, as compared to EBF infants. This finding would suggest formula feeding might allow infants a greater opportunity to express their genetic risk towards rapid weight gain. Perhaps, given i) the greater ease and speed with which milk can be delivered to infants through a bottle as compared to the breast[145] or ii) the nutritional variations between breast and formula milk that may influence appetite regulation[109]. The common association between formula feeding and rapid growth may be confounded by infants' genetic liabilities over early weight development. However, I did not find significant differences in the heritability estimates for RIWG by IFM. Hence, one might postulate that the ability for feeding methods and behaviours to moderate the expression of genetic risk for weight development might emerge in later in childhood or with alternative aspects of feeding beyond infancy. However, I did observe a non-significant trend, such that infants fed with predominantly formula milk showed slightly higher heritability for RIWG (A=0.39, CI=0.11, 0.44) than EBF infants (A=0.26, CI=0.25, 0.54). As an absolute difference was present - the current twin model might therefore have been underpowered to detect such differences in genetic and environmental influences over RIWG between feeding modalities. Hence, further alternative twin cohorts with appropriate measures and perhaps with larger samples are needed to confer the lack of GE interaction between IFMs and infant weight development within Gemini. Moreover, as GE interactions have been demonstrated between PFPs and weight development in later childhood[113,114] future of how PFPs may moderate the expression of genetic risk is also warranted.

In summary, whilst Studies 1 and 2 demonstrated reciprocity between IFMs and measured infant weight development – this reciprocity did not extend to infants' genetic liability for weight development. Infant feeding practices may be under greater influence from shared environmental influences, such as socioeconomic and environmental exposures, or crucially, parental perceptions of their child's weight development.

7.2.2 Merits and Limitations of Thesis Part One

The findings from Studies 1 to 3 using the Gemini Twin cohort must be considered alongside several merits and limitations. Whilst these are discussed in greater detail under each chapter, the broader merits and limitations across Studies 1-3 are presented here.

7.2.2.1 Merit #1: Repeated Measures of Feeding and Weight Within the Gemini Twin Sample

The Gemini sample offers some of the most detailed measures of IFM alongside reliable and repeated measures of infant weight gain of any UK birth cohort to date.[121] These detailed measures enabled the development of a detailed 7-group measure of IFMs, providing the unique opportunity to identify infants across feeding methods (e.g. formula feeding versus breastfeeding) and feeding modalities (e.g. breastfeeding from the breast versus from a bottle). Moreover, this measure was able to narrow in on a group of infants who were fed with expressed breastmilk in the first 3-months of life, offering a unique opportunity to establish whether bottle feeding with expressed breastmilk might place infants at greater risk of RIWG. In addition, the detailed measurement of timing of formula milk introduction allowed the second study to disentangle growth trajectories prior to and following the introduction of formula milk. To my knowledge, this is the first such study to disentangle infant growth relative to the exact week at which formula milk was introduced, which is essential to shed light on bi-directionality and generate causal inference in the relationship between feeding and growth outcomes. Secondly, Gemini offers a rich set of repeated growth measurements across the first year of life. This allowed the thesis to explore the association between feeding modalities on infant growth *trajectories*, as opposed to crude measures of RIWG (e.g. change in weight for age z-score from birth to 12-months) which are commonly used by other studies. Finally, 96.4% of weight measurements at 3 months in Gemini had been taken by a healthcare professional[52] as compared to parent-reported measurements of weight – where errors in measurement can lead to significant errors in weight data. Finally, at baseline, Gemini collected comprehensive measures of infant health characteristics, parental attributes, and the wider socioeconomic environment which could be accounted for carefully in the epidemiological analyses undertaken.

7.2.2.2 Merit #2: Leveraging the Twin Design to Garner Greater Causal Inferences

Finally, perhaps the greatest merit was the ability to triangulate bidirectional epidemiology with the discordant and classical twin designs to make causal inferences in relationships between IFMs and infant growth.[35] Firstly, using the discordant twin design I was able to observe the IFM-weight gain association while controlling for confounding from all environmental factors shared entirely by co-twins, such as family socioeconomic position – a pervasive limitation given the social patterning of both breastfeeding and infant and child weight development. However, whilst the twin design can remove statistical confounding from shared environmental exposures, it cannot remove confounding from non-shared environmental factors – such as differences in infant weight gain between twins.[138] However, in this way, the DT offered a unique and powerful opportunity to explore the unique characteristics of infants that might lead to different feeding exposures within a twin pair. Once again, removing social confounding from shared environmental sources. Therefore, the DT design was critical to exploring parent-child reciprocity in studies 1 and 2 of this thesis. Ultimately, the twin design of Gemini allowed me to undertake a highly novel study of GE interplay in infancy, as genetic influences and interactions are rarely considered in the relationship between infant feeding and infant growth.

7.2.2.3 Limitation #1: Weight Development of Twins Versus Singletons

The growth of twins is not the same as that of singletons which should be considered when interpreting the findings. Twins are, on average, born at a lower birthweight due to foetal restriction, and thus have a greater propensity to show RIWG or 'catch up growth'.[21,25] This is apparent in Gemini, with high rates of RIWG (change in weight-for-age z-score > 0.67) from birth to both 3- (55.2%) and 12-months of age (79.2%) To help improve the generalizability of the findings to singleton infants, we excluded pre-term infants (<36 weeks) from the analyses in Studies 1 and 2 and adjusted for confounding influences such as time spent in neonatal specialist care. This also aids in comparability to the UK-WHO Growth Reference Data which does not include growth data for infants born <32 weeks gestation, as only 1.5% of births occur before 32 weeks gestation. However, it is worth considering that the UK-WHO growth references do not adjust for gestational age for infants born to term (>37 weeks) due to inconsistent adjustment for gestation from healthcare professionals and resulting computation errors identified when the growth references were derived. Whilst infants born <36 weeks were excluded from the present analyses, many of the Gemini infants were born at 36 (n=708, 14.80%), 37 (n=930, 19.44%), and 38 weeks of age (n=1290, 26.96%) making this an important limitation to consider.

Moreover, the thesis also utilised trajectories of weight for age z-scores, as opposed to crude measures of RIWG such as change z-score from birth to 12-months, to detect more variation in weight gain trajectories. Finally, whilst RIWG may be overrepresented in Gemini, the *relationship* between infant feeding modalities and infant growth itself is unlikely to vary between singletons and twins. Hence, the current findings nevertheless shed important and novel light on parent-child reciprocity in infant feeding, given the opportunities provided by twins such as the DT model. These novel insights should therefore be further explored in singleton samples.

7.2.2.4 Limitation #2: Limited Diversity of The Gemini Sample

The Gemini sample includes a high proportion (61.8%) of high SES families and was largely white (93.0%). To assess their generalisability, the current findings should be explored in more ethnically, culturally and socioeconomically diverse samples. For example, the finding that formula feeding is a response to slower infant weight gain might be less prevalent in lower SES samples, where wider socioeconomic barriers to breastfeeding might be a stronger determinant of formula milk introduction. This is important to consider as a higher proportion of our sample for the LMMs (Study Two) exploring reciprocity in IFMs was of a high SES (76.9%) as compared to the baseline sample of Gemini (61.8%). Nonetheless, given the rarity of the detailed measures of infant feeding and repeated growth measures that Gemini offers – the present findings are still important to the field of infant feeding if considered in line with the families that they represent. Every effort was taken to adjust for varied measures of socioeconomic standing – using comprehensive composite measures of socioeconomic advantage[122] – across the epidemiological analyses presented in Studies 1 and 2.

7.2.2.5 Limitation #3: Self-Reported and Retrospective Measures Infant Feeding

Measurement error and recall biases are an important consideration given the maternal self-reports used to derive the measures of IFM. In Gemini, when their infants were approximately 8-months old (baseline) mothers were asked to recall the feeding methods they used in the first 3-months of life. Given the retrospective nature of this measure, recall errors might be prevalent. Similarly, given the highly emotive nature and stigma surrounding infant feeding, social desirability may have resulted in an underreporting of formula feeding. Moreover, as only mothers were asked to recall feeding methods, feeding modalities more likely to be provided by partners or fathers (e.g. expressed breastfeeding or formula feeding supplementation) may be underrepresented. Nonetheless, as infant feeding is a highly emotive and 'front-of-mind' aspect of infant care, recall errors might be less prevalent for feeding than other aspects of infant care. Moreover, it is worth mentioning that whilst the seven categories of IFM provide much detail, it is less nuanced regarding the ratios of formula to breastfeeding captured within some of these groups. Hence, whilst two infants may have both received 'breastfeeding and formula feeding' the ratio of these two methods might differ significantly, with one twin being fed 'mostly breast and some formula' and the other 'mostly formula and some breast'.

7.2.2.6 Limitation #4: Experiences of Feeding Twins Versus Singletons

Although the twin design offers powerful tools to explore reciprocity towards infant characteristics and GE interplay, feeding practices made in the context of caring for twins may differ from practices made in the context of caring for singletons.[21,154] Twins are born earlier and at lower birthweight[21], and therefore are more likely to experience specialist care and health complications in the early weeks of infancy. Overall, the proportion of having ever breastfed is similar between Gemini (77% of the sample) and nationwide data from the 2010 UK Infant Feeding Survey (IFS), which reported 69% and 81% for multiple and singleton infants, respectively.[52,142] However, the IFS demonstrates that twins are more likely to have formula milk by one week of age.[142] This is observable in Gemini, as 74.4% of infants fed with formula milk during the first 3-months were introduced to formula milk in the first week of life. This led to a smaller sample (N=450), than might be available in a singleton sample, for my reciprocity LMMs which required infants to be exclusively breastfed for at least 2-weeks to assess whether formula provision might be a response to infant growth. Moreover, breastfeeding two infants at the same time, as opposed to one, might be more taxing and demanding for mothers (logistically, physically, emotionally) leading to greater formula milk supplementation. Finally, regarding reciprocity, slower weight gain in a twin may be more salient than that in a singleton, because a direct comparison can be made with their co-twin. In addition, slower weight gain may be particularly concerning for a twin versus a singleton as they are born earlier and smaller, and have more health complications following birth. These factors may result in a lower threshold for parental intervention (i.e. introducing formula milk) towards slower weight gain. Hence, the presence of parental reciprocity in feeding should be corroborated by singleton studies, such as was demonstrated in the Cambridge Baby Growth Study.[70] However, to date, cohorts with sufficiently detailed measures of infant feeding, and repeated measures of weight development are rare. Despite experiential differences in feeding twins, twins offer a powerful and unique opportunity to evaluate reciprocity in infant feeding (e.g. through the discordant twin design) which are not possible with singleton samples.

7.2.2.7 Limitation #5: Assumptions of the Twin Design

In relation to exploring GE interplay in Study 3, there are several limitations of the twin design that need be considered. Twin modelling relies on the assumptions that MZ and DZ twins share their home and social environments to the same extent (so-called the equal environments assumption (EEA)).[159] However, MZ twins may be treated more similarly, or may select into more similar environments than their DZ counterparts . If environmental factors increase similarity between MZ twins, the result is inflated heritability estimates (which are estimated by modelling the difference between MZ and DZ similarity). Moreover, regarding the DT design, whilst twin pairs share the same socioeconomic standing (which is therefore removed as a confounder), there may be subtle ways in which they differ in their own unique experiences of their home and social environments, which can bias the assumptions of DT comparisons. However, given that the current DT comparisons were made within the first year of life, differential experiences of the socioeconomic environment are likely to be smaller than in later childhood and therefore this bias is likely to be minimal.

Finally, a consideration of DT comparisons is that parents are unlikely to feed their twins differently unless there is an important difference between the twins that occurred in early life – such as one twin experiencing an illness or weight faltering. For this reason, DT comparisons can remove confounding from the shared environment, but not unique individual characteristics and experiences that might contribute both to discordance in feeding and discordance in weight development. Nonetheless, as aforementioned, this limitation is also a merit, as individual characteristics that lead to discordant infant feeding can be exposed by twin approaches such as the DT design. This approach was of merit to the current thesis in exploring parent-child reciprocity in infant feeding, providing strong evidence that formula milk supplementation is a response to early infant weight development, independent of shared environmental factors.

7.3 Discussion of Findings from Part Two of the Thesis

7.3.1 Implications for Theory, Policy, and Practice

For the final chapter, I led the development of the protocol for the Baby Responsive Intervention for Growth & Health Tracking (BRIGHT), a novel digital intervention targeting responsive bottle-feeding practices to promote healthy growth amongst formula fed infants through the Baby Buddy app. Using the PBA for intervention development[209], I describe two stages of development, utilising PPIE co-development with caregivers, the BCW and COM-B model for intervention component development, as well as qualitative research with target users to optimise the prototype in line with the APEASE framework. In summary, caregivers deemed the resulting prototype acceptable, engaging, and feasible to implement for families. Following this initial stage of prototype development, a formal evaluation of the BRIGHT intervention is planned (e.g. randomised controlled trial or 'real world' evaluation), which has the potential to gather rigorous evidence regarding relationships between infant feeding and infant growth and development. Following this evaluation, BRIGHT has potential to be rolled out nationally on the Baby Buddy app, at no cost to the user or health care providers. BRIGHT therefore is ideally positioned to support thousands of caregivers across the UK as well as generate important and rigorous evidence as to how feeding behaviours in infancy may influence child growth. Caregivers involved in the PPIE and qualitative research reported several findings and experiences that are useful to reflect on for future research and practice.

7.3.1.1 The Dearth of Formula Feeding Support & Pervasive Surrounding Stigma

In line with previous qualitative research with caregivers[171,175], the BRIGHT PPIE panel and qualitative research participants highlighted the dearth of comprehensive formula feeding guidance available to caregivers in the UK from reputable sources. Caregivers reported turning to less reputable sources, such as formula milk company websites and social media, to access the guidance they required. This clearly emphasizes the need to provide families with necessary support and guidance around formula feeding, as they make up the majority of families in the UK.[142] Moreover, given my finding (in Study 1) that bottle-feeding mechanisms and behaviours might be an important contributor to RWG amongst formula fed infants, there may be an important opportunity to lower this risk with provision of evidence-based bottle-feeding guidance. This opportunity has scarcely been considered across the infant feeding literature, although few trials seeking to promote responsive bottle feeding such as Baby Milk, INSIGHT, and NOURISH have demonstrated benefits for infant growth outcomes.[64,163,183] Therefore, further studies should seek to systematically explore these bottle-feeding behaviours and mechanisms to inform the scope of infant feeding interventions such as BRIGHT. Moreover, given the complex interactions between infant weight gain, feeding modalities, and feeding practices demonstrated in part one of the thesis – experimental designs such as BRIGHT might be particularly well placed to better disentangle cause and effect in relationships between feeding practices and growth outcomes than possible with observational studies. Moreover, there is an enormous opportunity to better support formula feeding families, which are the majority of infants in the UK, by offering parents comprehensive and evidence-based responsive and comprehensive feeding guidance. Based on my findings (Study 1) I hypothesise that such provision might result in more favourable weight trajectories amongst formula fed infants. This hypothesis needs to be tested in future research.

Secondly, the BRIGHT caregivers reported significant levels of stigma attached to formula feeding which influenced their feeding experiences and well-being. This stigma was largely a result of experiences with healthcare professionals, cultural narratives around formula feeding, and the lack of support and reputable guidance available to formula feeding parents in the UK. Provision of supportive formula feeding guidance therefore carries the potential to both support infant health and support caregiver mental health by reducing the perceived judgement and guilt that some parents feel (and the mental health ramifications of this) as a result of formula feeding their baby. Future studies should therefore explore the potential benefit of formula feeding guidance and more inclusive infant feeding policies on parental mental health and wellbeing. Importantly, the BRIGHT PPIE panel and qualitative participants expressed that formula feeding guidance must be sensitive in tone and consider the pervasive stigma that surrounds formula feeding in their delivery. This will ensure that efforts seeking to support formula feeding families do not inadvertently contribute to stigma or negative emotions that may already be affecting caregivers.

7.3.1.2 Responsive Formula Feeding or Feeding to the Guidelines?

Third, I observed an important friction between offering caregivers responsive feeding guidance (i.e. a child led approach to feeding) with provision of formula milk intake guidelines (i.e. a parent led approach to feeding) which was noted by many caregivers. Therefore, it is important to recognise and communicate to parents that the recommended daily intakes of formula milk provided by WHO and UK guidelines and formula companies are not the recommended amounts for *every* baby, as they are based on estimated average requirements according to age and sex.[249] Whilst it is important to provide this *guideline* to caregivers, it was deemed important for BRIGHT to focus on responsive feeding alongside this provision. The guidelines were therefore offered as a useful 'starting point', but ultimately BRIGHT encouraged responsive feeding such that they did not pressure or restrict their infant if they did not meet the recommended amounts. Whilst this was considered appropriate and sensible by the BRIGHT PPIE panel and research participants, they expressed that this nuance could be confusing for parents if not explained carefully. Moreover, many parents reflected that responsive feeding might be more challenging for some parents to follow than others, depending on their infant's characteristics. For example, following the advice to not pressure a child might be more challenging for infants who are growing slower than expected or express a small appetite. Similarly, parents reflected that following responsive feeding guidance to not restrict a child's milk intake and respond to their hunger cues might result in overfeeding if the child has a heartier appetite. It is therefore important for future interventions to convey that responsive feeding is not simply 'indulgent feeding' to all child cues but guides parents to appropriately respond also using feedback on their child's growth pattern. Moreover, it would be of merit to investigate the implications of responsive feeding guidance for children of varying appetitive traits.

7.3.1.3 Tailored and Interactive Infant Feeding Interventions Are Promising

One of the aims of BRIGHTs co-creation work with caregivers was to explore whether content that is tailored to an infant's individual characteristics (e.g. growth) is an acceptable and useful approach for infant feeding interventions. This aim was informed by part one of the thesis, which demonstrated the presence of parent-child reciprocity in infant feeding. I hypothesised that feeding guidance which is tailored to a child's unique profile might be more engaging, useful, and acceptable to caregivers – although these assumptions needed to be tested empirically. In line with this hypothesis, BRIGHT's PPIE panel and research participants found feeding guidance tailored to their infant's growth highly novel, useful, and acceptable, if delivered in a supportive tone. For example, receiving 'top feeding tips' tailored to their infant's growth centile was deemed helpful and easy to implement. Moreover, caregivers appreciated BRIGHT's acknowledgement that 'every baby is unique' and felt that the recognition of individual differences across infants improved the acceptability and salience of the resources. However, future interventions such as BRIGHT should seek to explore the potential for *tailored* digital feeding interventions to change parental feeding behaviours.

Moreover, caregivers expressed the value of interactive tools in digital interventions seeking to support infant feeding and growth (e.g. infant feeding trackers and growth chart features). Whilst we believe such tools might be imperative for effective behaviour change, in that they can enable numerous evidence-based BCTs such as self-monitoring, goal setting, and feedback on behaviours, they are expensive to develop and their efficacy needs to be established. For instance, it would be of merit to investigate how the currently available infant feeding trackers, commonly used by both breastfeeding and formula feeding caregivers, might influence responsive feeding practices as well as infant weight outcomes. It has been suggested that feeding trackers, which parents desire, might inadvertently deter a responsive approach to feeding and encourage more pressuring or restrictive infant feeding of infants who differ from average requirements of formula milk intake.[182] Hence, this interaction between self-monitoring and responsive feeding behaviour would be of merit to investigate further.

7.3.2 Merits and Limitations

7.3.2.1 Merits

BRIGHT's development benefitted from applying the PBA, as it implemented a theory-based and rigorous approach to integrating user perspectives into the digital prototype.[209] Specifically, by integrating perspectives of target-users into every stage of prototype development the PBA helps to ensure that digital interventions are attractive, persuasive, and feasible to implement.[209] Moreover, in line with the PBA, we implemented systematic intervention development frameworks such as a theory of change model, rapid tabulation of PPIE feedback, as well as the APEASE criteria to help mitigate the research team's assumptions and potential researcher biases that might influence the development of BRIGHT.[222] Moreover, it is a strength that BRIGHT involved a diverse set of target user caregivers in the PPIE panel to co-create the prototype – including both mothers and fathers. This helped ensure that BRIGHT's resources were sensitive to the pervasive stigma that surrounds formula feeding, and that they were not unintentionally stigmatising for families living in different contexts. Moreover, Best Beginnings' Baby Buddy app is widely adopted across the UK (with ~7,000 caregivers downloading it every month) and has a large representation of families living in financial hardship.[207] Therefore, we felt it important that our PPIE panel represent a variety of families, to ensure that BRIGHT is acceptable and practical for families living in varying contexts. Finally, alongside PPIE co-creation, we implemented a qualitative 'In the Wild' study to test and optimise the BRIGHT prototype 'package' within the day-to-day life of families. Hence, we were able to ascertain the acceptability and engagement with the BRIGHT prototype after it was *applied* to day-to-day behaviours, as opposed to solely the hypothetical acceptability and feasibility of the isolated and static resources.

7.3.2.2 Limitations

First, the current BRIGHT prototype is limited in its provision of 'interactive' digital tools, therefore limiting the breadth of BCTs that could be integrated into the protype. For example, PPIE co-creation with caregivers identified a 'formula feeding tracker' as a promising and useful tool to be delivered within BRIGHT, which would also allow for BCT's such as goal setting and self-monitoring to be implemented, and for caregiver self-efficacy to better be improved. However, we were not able to co-develop a functional prototype for a formula feeding tracker as development costs exceeded the available funds. Together with Best Beginnings, we are planning to seek funding for the development and testing of such a functional add-on. In addition, while we recruited a diverse PPIE panel to co-create BRIGHT, our 'In the Wild' optimisation study consisted only of mothers who were highly educated. We postulate that this was result of the high demands of participation in this study, as we recruited through a diverse sample of current Baby Buddy users. Therefore, the extent to which these results can be generalised to caregivers living in deprivation, and those with lower educational attainment, partners and fathers, and non-white ethnicity and other minority groups such as LBGTQIA+ families, needs to be further evaluated. Finally, it is important to reflect on sampling biases in the BRIGHT PPIE panel and the research participants in the 'In the Wild' study. Whilst we recruited through Baby Buddy networks, to mirror the characteristics of Baby Buddy users to which BRIGHT would eventually be delivered to, it remains possible that more health-conscious and tech-literate parents are likely to have participated. Future evaluation work and piloting therefore seeks to evaluate the BRIGHT prototype in a larger, more diverse sample of Baby Buddy users than those who participated in PPIE and the qualitative sample.

7.4 Concluding Statement

Part one of this thesis (Studies 1-3) triangulated bidirectional epidemiology with the discordant twin and classical twin designs to highlight parent-child reciprocity in infant feeding and weight development within the Gemini Twin Study. First, IFMs were shaped by early infant weight gain – as formula milk supplementation was, in part, a response to slower weight gain and maternal concern for lower infant weight gain during the early weeks of infancy. However, infant weight gain was also shaped by IFMs. Formula feeding, in isolation or combination with breastmilk, promoted more rapid weight gain after its introduction. This 'two-way street' of feeding is scarcely considered in previous research, practice, and policy related to the first 1,000 days period. Therefore, I propose that future studies should seek to disentangle how infant characteristics might shape both *what* (e.g. infant feeding methods) and *how* (e.g. parental feeding practices) infants are fed. These studies will contribute to a richer theoretical understanding of parent-child reciprocity within infancy and greater understanding of causal inference in the relationship between IFMs and infant weight development. Moreover, I propose that future infant feeding interventions and policies should consider the dynamic and bi-directional interactions between caregiver and infants demonstrated by the present results. For instance, interventions seeking to promote longer durations of breastfeeding or more responsive feeding practices may benefit from recognising how these practices themselves may be shaped by the unique characteristics of children that emerge from early infancy.

In addition, my findings implicated bottle-feeding as a mechanism that may, in part, promote RIWG after the introduction of formula milk. I therefore propose that research and policies aiming to support health weight development in infancy should also focus on the *how* of milk feeding (e.g. responsive bottle-feeding practices), as well as the *what* of milk feeding (e.g. formula feeding versus breastfeeding through breastfeeding promotion efforts). Whilst I found no evidence of GE interplay between infant feeding and weight gain in the Gemini study, future studies with greater samples and sufficient infant feeding measures should explore GE interactions and correlation further. Finally, part two of my thesis presented the protocol for BRIGHT, a novel digital intervention targeting responsive bottle-feeding practices to promote healthy growth amongst formula fed infants. The development of BRIGHT highlighted the significant opportunity to support formula feeding families with comprehensive, impartial, and evidence-based feeding guidance. This guidance carries potential to support healthy infant weight development, given the findings from part one of the thesis. However, I also propose that this guidance carries potential to support parental mental health and wellbeing, given the pervasive stigma attached to formula feeding and lack of reputable formula feeding resources available to families. A rigorous future evaluation of BRIGHT will also help to provide more rigorous experimental evidence establishing greater cause and effect in the relationship between infant feeding practices and infant weight development.

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Appendices

Appendices for Chapter Three. Study 1: Infant Feeding Modalities and Infant Weight Gain Trajectories Across the First Year of Life

Appendix I. Table of Estimates for Unadjusted Linear mixed effects models (LMMs) exploring the effect of infant feeding modalities on weight for age z-score trajectories across the first 12-months of life

	Weight-for-age Z-score Trajectories 0-12 Months[a] (n= 2,688)			
Infant Feeding Modality (3-Group Measure)	N	M (SD) Number of Weight Measurements	β	95% CI
IFM Term; Weight-for-age Z-score at Baseline[x]				
Exclusively Breastfed (ref)	425	23.06 (9.04)	-	-
Mixed Fed	1,288	22.45 (10.39)	-0.16	-0.54, .22
Formula Fed	975	19.35 (8.45)	0.04	-0.08, 0.17
IFMxTime Term; Linear Rate of Change in Z-score per week[d]				
Exclusively Breastfed[b] (ref)			-	.-
Mixed Fed			0.04***	0.02, 0.04
Formula Fed			0.04***	0.02, 0.04
IFMxTime^2 Term; Quadratic Rate of Change in Z-score Per Week[d]				
Exclusively Breastfed[b] (ref)			-	-
Mixed Fed			-.00069***	-.00091, -.00046
Formula Fed			-.00073***	-.00097, -.00049
Variance				
Within-person			0.00	0.00, 0.00
In initial status			0.17	0.14, 0.20
In rate of change			-0.00	-0.01, -0.00
Infant Feeding Modality (7-Group Measure)				

IFM Term; Z-score at Baseline				
Exclusively Breastfed[b] (ref)	401	23.31 (9.09)	-	
Breastfed and Expressed Fed	71	20.10 (7.41)	-0.35***	-0.61, 0.10
Expressed Fed	54	22.85 (9.92)	0-.63**	-0.95, -0.35
Breast and Formula Fed	907	22.38 (9.68)	-0.11	-0.24, 0.02
Breast, Formula and Expressed	141	23.38 (14.82)	-0.12	-0.32, 0.07
Formula and Expressed Fed	139	22.43 (9.88)	-0.30**	-0.49, -0.11
Formula Fed	975	19.35 (8.45)	-0.002	-0.14, 0.13
IFMxTime Term; Linear Rate of Change in Z-score per week[d]				
Exclusively Breastfed[b] (ref)			-	-
Breastfed and Expressed Fed			0.04***	0.01, 0.06
Expressed Fed			0.06***	0.03, 0.08
Breast and Formula Fed			0.04***	0.03, 0.05
Breast, Formula and Expressed			0.04***	0.03, 0.06
Formula and Expressed Fed			0.04***	0.02, 0.06
Formula Fed			0.04***	0.02, 0,05
IFMxTime^2 Term; Quadratic Rate of Change in Z-score Per Week[d]				
Exclusively Breastfed[b] (ref)			-	-
Breastfed and Expressed Fed			-0.00057**	-0.00104, -0.0009
Expressed Fed			-0.00103***	-0.00153, -0.0005374
Breast and Formula Fed			-0.00064***	-0.00089, -0.0003913
Breast, Formula and Expressed			-0.00088***	-0.00125, -0.0005209
Formula and Expressed Fed			-0.00078***	-0.00115, -0.0004068
Formula Fed			-0.00075***	-0.00099, -0.0005035
Variance				
Within-person			0.00	0.00, 0.00
In initial status			0.16	0.13, 0.19
In rate of change			-0.01	-0.01, -0.00

Weight for age Z-score; Calculated using UK-WHO growth refence data adjusting for age, sex, and gestational age for each weight measurement.
3-Group Infant Feeding Method; Breastfed: Fed breast milk from the breast or from expressed milk; Expressed Milk Fed; Fed with primarily expressed milk from a bottle or equal amount of breastmilk from the breast and expressed milk from a bottle; Mixed-Fed: Fed with a combination of breast milk from the breast, expressed milk in a bottle, and formula milk; Formula Fed: Fed with formula milk
7-Group Infant Feeding Modality, Exclusively Breastfed: Fed exclusively breast milk from the breast; Breastfed and Expressed Fed: Fed with a combination of breast milk from the breast and expressed milk in a bottle; Expressed Fed: Fed with expressed milk in a bottle; Breastfed and Formula fed; Fed with a combination of breast milk from the breast and formula milk; Breast, Formula and Expressed: Fed with a combination of breast milk from the breast, expressed milk in a bottle, and formula milk; Formula and Expressed Fed: Fed with a combination of expressed milk from a bottle and formula milk; Formula Fed: Fed with formula milk
[c]Baseline: Occasion 2 of weight measurement; mean = 3.18 week (SD=2.11)
[d]Rate of Change in Z-score Modelled from Baseline (m=3.18 weeks) to 52 weeks of age
*** p < 0.001; ** p < 0.01; * p < 0.05.
[a] The within-person variance is the overall residual variance in weight that is not explained by the model. The initial status variance component is the variance of individuals' intercepts about the intercept of the average person. The rate of change variance component is the variance of individual slopes about the slope of the average p

Appendix II. Table of Estimates for Adjusted Linear mixed effects models (LMMs) exploring the effect of infant feeding modalities on weight for age z-score trajectories across the first 12-months of life

	Weight-for-age Z-score Trajectories 0-12 Months[a] (n=1998)			
Infant Feeding Modality (3-Group Measure)	N	M (SD) Number of Weight Measurements	β	95% CI
IFM Term; Weight-for-age Z-score at Baseline[x]				
Exclusively Breastfed[b] (ref)	317	23.06 (9.04)	-	-
Mixed Fed	967	22.45 (10.39)	-0.11	-0.25, .17
Formula Fed	714	19.35 (8.45)	0.02	-0.12, 0.17
IFMxTime Term; Linear Rate of Change in Z-score per week[s]				
Exclusively Breastfed[b] (ref)			-	.-
Mixed Fed			0.03***	0.02, 0.05
Formula Fed			0.03***	0.02, 0.04
IFMxTime^2 Term; Quadratic Rate of Change in Z-score Per Week[d]				
Exclusively Breastfed[b] (ref)			-	-
Mixed Fed			-.00059***	-.00086, -.00032
Formula Fed			-.00063***	-.00091, -.00035
Variance				
Within-person			0.00	0.00, 0.00
In initial status			0.16	0.13, 0.20
In rate of change			-0.00	-0.01, -0.00
Infant Feeding Modality (7-Group Measure)				
IFM Term; Z-score at Baseline				
Exclusively Breastfed[b] (ref)	297	23.31 (9.09)	-	-1.63, 1.61
Breastfed and Expressed Fed	57	20.10 (7.41)	-0.41**	-0.68, -0.15
Expressed Fed	39	22.85 (9.92)	-0.49***	-0.83, -0.14
Breast and Formula Fed	674	22.38 (9.68)	-0.11	-0.26, 0.03
Breast, Formula and Expressed	120	23.38 (14.82)	-0.13	-0.34, 0.07
Formula and Expressed Fed	97	22.43 (9.88)	-0.24*	-0.47, -0.02
Formula Fed	714	19.35 (8.45)	-0.0	-0.16, 0.14

IFMxTime Term; Linear Rate of Change in Z-score per week[d]		
Exclusively Breastfed[b] (ref)	-	-
Breastfed and Expressed Fed	0.03**	0.00, 0.06
Expressed Fed	0.04**	0.01, 0.07
Breast and Formula Fed	0.03***	0.02, 0.05
Breast, Formula and Expressed	0.04***	0.02, 0.06
Formula and Expressed Fed	0.03**	0.00, 0.05
Formula Fed	0.03***	0.02, 0.05
IFMxTime^2 Term; Quadratic Rate of Change in Z-score Per Week[d]		
Exclusively Breastfed[b] (ref)	.00013	-.00010, .00038
Breastfed and Expressed Fed	-.00053***	-.00105, -.00002
Expressed Fed	-.00082***	-.00141, -.00024
Breast and Formula Fed	-.00056***	-.00085, -.00027
Breast, Formula and Expressed	-.00080***	-.00120, -.00040
Formula and Expressed Fed	-.00060***	-.00102, -.00018
Formula Fed	-.00064***	-.00092, -.00035
Variance		
Within-person	0.00	0.00, 0.00
In initial status	0.16	0.13, 0.20
In rate of change	-0.00	-0.00, -0.00

Weight for age Z-score; Calculated using UK-WHO growth refence data adjusting for age, sex, and gestational age for each weight measurement.

[a] Adjusted for birthweight, zygosity, sex, gestational age, days spent in specialist care, maternal age at delivery, mode of delivery, maternal BMI, maternal ethnicity, maternal marital status, maternal smoking during pregnancy, gestational diabetes, composite socioeconomic position, introduction of solid foods, and clustering within families

[c]Exclusively Breastfed: Fed exclusively breast milk from the breast; Breastfed and Expressed Fed: Fed with a combination of breast milk from the breast and expressed milk in a bottle; Expressed Fed: Fed with expressed milk in a bottle; Breastfed and Formula fed; Fed with a combination of breast milk from the breast and formula milk; Breast, Formula and Expressed: Fed with a combination of breast milk from the breast, expressed milk in a bottle, and formula milk; Formula and Expressed Fed: Fed with a combination of expressed milk from a bottle and formula milk; Formula Fed: Fed with formula milk

[c]Baseline: Occasion 2 of weight measurement; mean = 3.18 week (SD=2.11)

[d]Rate of Change in Weight Modelled from Baseline (m=3.18 weeks) to 52 weeks of age

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.

[a] The within-person variance is the overall residual variance in weight that is not explained by the model. The initial status variance component is the variance of individuals' intercepts about the intercept of the average person. The rate of change variance component is the variance of individual slopes about the slope of the average person.

Appendix III. Parameter Estimates of Linear Mixed Effects Models Comparing Weight for Age Z-Score Trajectories to 12-months of age Between Twin Pairs Discordant for Infant Feeding Modality (n=108 twin pairs)

	Weight-for-age Z-Score Trajectories [0-12 Months]	
	Estimate (β)	95% CI
(Intercept)	-.08	-.34, 0.16
Baseline Weight of Twin Fed with Higher risk IFM	-.00	-.03, 0.02
Increase in Weight over Time of Twin Fed With Higher Risk IFM (Twin*Age_Wt)	-.01	-.29, 0.08
Increase in Weight over Time of Twin Fed With Higher Risk IFM (Twin*Age_Wt^2)	.00	-.00, .00
N Total (Twin Pairs)		108

Weight for age Z-score; Calculated using UK-WHO growth refence data adjusting for age, sex, and gestational age for each weight measurement.
[a]Twin Pairs Discordant in Infant Feeding Modalities; Exclusively Breastfed, Breastfed and Expressed Fed, Expressed Fed, Breastfed and Formula fed, Breast, Formula and Expressed, Formula and Expressed Fed, Formula Fed: Fed with formula milk. Infant Fed with More bottle, whether through expression or formula milk categorized as 'more bottle' fed twin
[c]Adjusted for; Difference in sex, difference in birthweight z-score, difference in days spent in specialist care, difference in timing of introduction to solid foods

Appendices for Chapter Four. Study 2: Parent-Child Reciprocity in Infant Feeding Modalities and Infant Weight Development Across the First Year of Life

Appendix IV. Results of Adjusted Logistic Regression Models Between Weight-for-age Change Z-score Prior to Introduction of Formula Milk or Concern for Low Weight Gain from 0-3 months and Infant Feeding Methods

Weight-for-age Change Z-score Prior to Introduction of Formula Milk (n=479)	N	Odds Ratio of Mixed or Formula Feeding	CI	P value
IFM Term				
Exclusively Breastfed[a] (ref)	353	-	-	
Mixed fed or Formula Fed	126	0.37***	0.24, 0.57	0.000
cons		.82	-1.33, 2.98	
Maternal Reported Concerned for Low Weight Gain from 0-3 Months (n=585)				
Exclusively Breastfed[b] (ref)	373	-	-	
Mixed fed or Formula Fed	212	1.75	0.97, 3.14	0.059
cons		149.30	2.141, 10410.6	

*** p < 0.001; ** p < 0.01; * p < 0.05.
Weight-for-age Change z-score Prior to Introduction of Formula Milk; Derived using: change in weight for age z-scores to the point of introduction of formula milk were calculated by subtracting an infant's birthweight z-score from their z-score at the start of the week in which formula was introduced (6-weeks for Breastfed Infants)
Maternal Reported concern for Low Weight Gain Between 0-3 Months; Measured by the question: 'Have you ever been concerned that your baby wasn't gaining enough weight?' with a follow up question asking mothers to specify the time at which they were concerned (0-3 months of age was the period of time included for the analyses).
Adjusted for birthweight, zygosity, sex, days spent in specialist care, maternal age at delivery, mode of delivery, maternal BMI, maternal ethnicity, maternal marital status, maternal smoking during pregnancy, gestational diabetes, composite socioeconomic position, introduction of solid foods, and clustering within families, gestational age + timing of introduction to formula (set at 6-weeks for BF infants) and clustering within families

Appendix V. Unadjusted Logistic Regression Models Between Weight-for-age Change Z-score Prior to Introduction of Formula Milk or Concern for Low Weight Gain from 0-3 months and Infant Feeding Methods

Weight-for-age Change Z-score Prior to Introduction of Formula Milk (n=596)	N	Odds Ratio of Mixed or Formula Feeding	CI	P value
IFM Term				
Exclusively Breastfed[a] (ref)	436	-	-	
Mixed fed or Formula Fed	160	0.52***	0.38, 0.71	<0.001
cons		.82	-1.33, 2.98	
Maternal Reported for Low Weight Gain from 0-3 Months (n=917)				
Exclusively Breastfed[b] (ref)	638	-	-	
Mixed fed or Formula Fed	279	1.34	.84, 2.12	0.216
cons		13.73	1.98, 95.02	

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.
Weight-for-age Change Z-score Prior to Introduction of Formula Milk; Derived using: change in weight for age z-scores to the point of introduction of formula milk were calculated by subtracting an infant's birthweight z-score from their z-score at the start of the week in which formula was introduced (6-weeks for Breastfed Infants)
Mother Concerned for Low Weight Gain Between 0-3 Months; Measured by the question: 'Have you ever been concerned that your baby wasn't gaining enough weight?' with a follow up question asking mothers to specify the time at which they were concerned (0-3 months of age was the period of time included for the analyses).

Appendix VI. Adjusted Logistic Regression Models Between Weight-for-age Change Z-score Prior to Introduction of Formula Milk or Concern for Low Weight Gain from 0-3 months and Infant Feeding Methods Excluding Expressed Fed Infants

Weight-for-age Change Z-score Prior to Introduction of Formula Milk (n=421)	N	Odds Ratio of Mixed or Formula Feeding	CI	P value
IFM Term[a]				
Exclusively Breastfed[a] (ref)	318	-	-	
Mixed fed or Formula Fed	103	0.34***	0.20, 0.56	<0.001
cons		6.55	0.04, 1035	
Maternal Reported for Low Weight Gain from 0-3 Months (n=518)				
Exclusively Breastfed[b] (ref)	339	-	-	
Mixed fed or Formula Fed	179	1.74	0.93, 3.23	0.080
cons		13.73	1.98, 95.02	

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.
[a]Infants fed with expressed breastmilk excluded from both Exclusively Breastfed and Mixed or Formula Fed infants
Weight-for-age Change Z-score Prior to Introduction of Formula Milk; Derived using: change in weight for age z-scores to the point of introduction of formula milk were calculated by subtracting an infant's birthweight z-score from their z-score at the start of the week in which formula was introduced (6-weeks for Breastfed Infants)
Mother Concerned for Low Weight Gain Between 0-3 Months; Measured by the question: 'Have you ever been concerned that your baby wasn't gaining enough weight?' with a follow-up question asking mothers to specify the time at which they were concerned (0-3 months of age was the period of time included for the analyses).
Adjusted for birthweight, zygosity, sex, days spent in specialist care, maternal age at delivery, mode of delivery, maternal BMI, maternal ethnicity, maternal marital status, maternal smoking during pregnancy, gestational diabetes, composite socioeconomic position, introduction of solid foods, and clustering within families, gestational age + timing of introduction to formula (set at 6-weeks for BF infants) and clustering within families

Appendix VII. Unadjusted Weight for Age Z-Score Trajectories Prior to and Following the Introduction of Formula Milk, Between Breastfed and Formula Fed Infants (N=951)

	Unadjusted Weight-for-age Z-score Trajectories Prior to the Introduction to Formula (N=951)				Unadjusted Weight-for-age Z-score Trajectories after the Introduction to Formula (N=951)			
	N	β	95% CI	P value	N	β	95% CI	P value
IFM Term; Weight for Age Z-Score at Baseline								
Exclusively Breastfed[b] (ref)	422	-	-		422	-	-	
Formula Fed	529	-.48	-0.84, -0.13	0.001***	529	-.09	-.23, 0.05	0.190
IFMxTime Term; Rate of Change in Weight for Age Z-score Per Week								
Exclusively Breastfed[b] (ref)	422	-	-		422	-	-	
Formula Fed	529	-0.08	-0.15, -0.01	0.01**	529	0.01	0.01, 0.02	0.02**
Cons		-0.38	-.57, -.19			0.16	0.03, 0.29	

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.

[B]Baseline for Prior to the Introduction to Formula was 6 weeks before to the introduction of formula milk (or birth for EBF infants), Baseline for Following the Introduction to Formula was week of formula milk introduction (or 6-weeks of age for EBF infants)

Exclusively Breastfed: Fed exclusively breast milk from the breast; or Fed with a combination of breast milk from the breast and expressed milk in a bottle or Fed with expressed milk in a bottle

Formula Fed; Fed with a combination of breast milk from the breast, expressed milk in a bottle, and formula milk; or predominantly formula milk introduced after 2-weeks of age

Appendix VIII. Adjusted Weight for Age Z-Score Trajectories Prior to and Following the Introduction of Formula Milk, Between Breastfed and Formula Fed Infants Removing Expressed Breastfed Infants (N=405)

	Adjusted Weight-for-age Z-score Trajectories Prior to the Introduction to Formula (N=405)				Adjusted Weight-for-age Z-score Trajectories after the Introduction to Formula (N=405)			
	N	β	95% CI	P value	N	β	95% CI	P value
IFM Term; Weight for Age Z-Score at Baseline								
Exclusively Breastfed[b] (ref)	309	-	-		309	-	-	
Formula Fed	96	-.66	-1.05, -0.27	0.001***	96	-.19	-.50, 0.10	0.197
IFMxTime Term; Rate of Change in Weight for Age Z-score Per Week								
Exclusively Breastfed[b] (ref)	309	-	-		309	-	-	
Formula Fed	96	-0.10	-0.17, -0.03	0.006**	96	0.02	0.01, 0.03	0.002**
Cons		0.47	-.02, 0.94			0.83	0.41, 2.08	

*** p < 0.001; ** p < 0.01; * p < 0.05.

[B]Baseline for Prior to the Introduction to Formula was 6 weeks before to the introduction of formula milk (or birth for EBF infants), Baseline for Following the Introduction to Formula was week of formula milk introduction (or 6-weeks of age for EBF infants)

Exclusively Breastfed: Fed exclusively breast milk from the breast; or Fed with a combination of breast milk from the breast and expressed milk in a bottle or Fed with expressed milk in a bottle

Formula Fed; Fed with a combination of breast milk from the breast, expressed milk in a bottle, and formula milk; or predominantly formula milk introduced after 2-weeks of age

Adjusted for birthweight, zygosity, sex, days spent in specialist care, maternal age at delivery, mode of delivery, maternal BMI, maternal ethnicity, maternal marital status, maternal smoking during pregnancy, gestational diabetes, composite socioeconomic position, introduction of solid foods, and clustering within families, gestational age + timing of introduction to formula (set at 6-weeks for BF infants) and clustering within families

Appendices for Chapter Five. Study 3: Gene-environment interplay in infant feeding modalities and infant weight development

Appendix IX. Fit statistics for GE Model Examining Heritability of Weight-for-age Change Z-Score from 0-12-months by Infant Feeding Modalities as Compared to the Scalar and Null models

Phenotypes	Base Model	Comparison	Estimated Parameters	-2LL	df	AIC	ΔLL	Δ χ^2 (df)	p-value
Weight-for-age Change Z-score at 12-months	GxE Model	-	8	5567.76	2143	5583.76	-	-	-
Weight-for-age Change Z-score at 12-months	GxE Model	Scalar Model (No GxE Model)	5	5569.15	2146	5579.15	1.38	3	0.71
Weight-for-age Change Z-score at 12-months	GxE Model	Null Model (No GxE Model)	5	5571.12	2146	5580.47	2.36	3	0.70

Abbreviations: ; df: degrees of freedom; -2LL: -2 log-likelihood; AIC: Akaike's Information Criterion, Δ χ^2: differenced in chi-square

Appendices for Chapter Six: A digital intervention to promote responsive formula-feeding and healthy growth in infancy; a protocol for the BRIGHT Intervention

Appendix X: List of Behaviour Change Techniques in Baby Milk Study

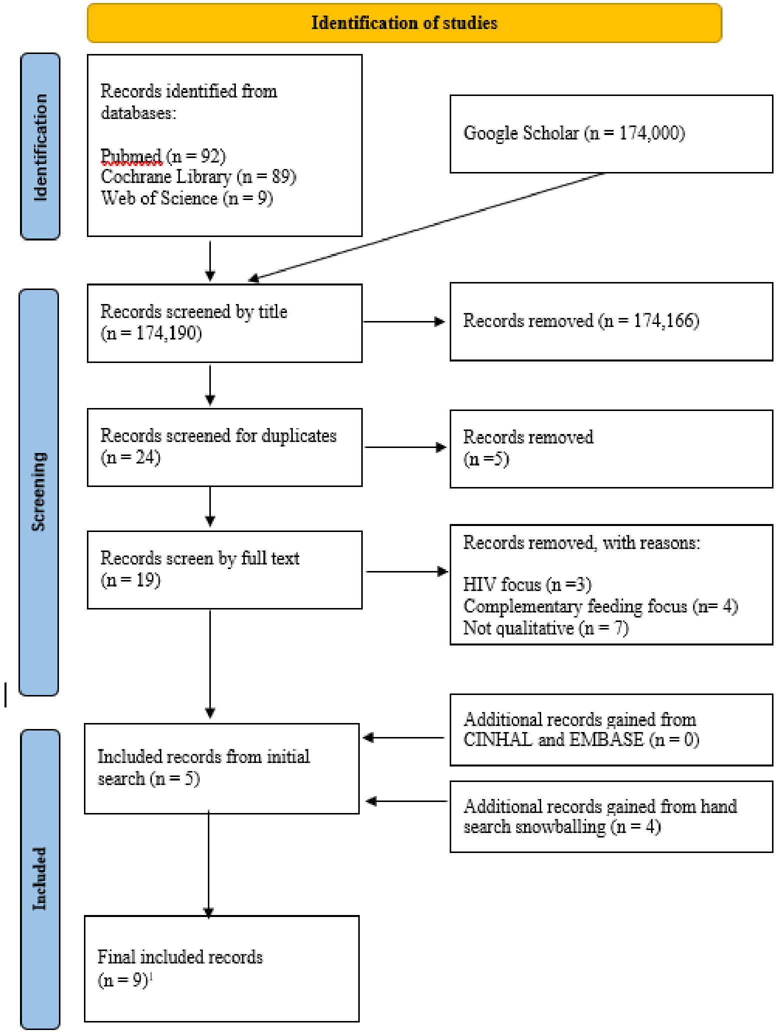
1. Information about health consequences (Natural Consequences)
2. Credible Source (Comparison of Outcomes)
3. Instruction on how to perform a behaviour (Shaping Knowledge)
4. Self-monitoring of outcome of behaviour (Feedback and Monitoring)
5. Self-monitoring of behaviour (Feedback and Monitoring)
6. Feedback on Behaviour (Feedback and Monitoring)
7. Salience of Consequences (Natural Consequences)
8. Habit Formation (Repetition and substitution)
9. Pros and Cons (Comparison of outcomes)
10. Restructuring the Physical environment (Antecedents)
11. Demonstration of the behaviour (Comparison of the Behaviour)
12. Behavioural practice and rehearsal (Repetition and Substitution)
13. Goal setting (behaviour) (Goals and Planning)
14. Goal Setting (outcome) (Goals and Planning)
15. Commitment (Goals and Planning)
16. Prompts / Cues (Associations)
17. Problem Solving (Goals and Planning)
18. Action Planning (Goals and Planning)
19. Discrepancy between current behaviour and goal (Goals and Planning)
20. Behaviour substitution (on the tin to WHO recommended) (Repetition and Substitution)
21. Information about others' approval (Comparison of Behaviour)
22. Social Support (Practical) (Social Support)
23. Social Support (Emotional) (Social Support)
24. Social comparison (Comparison of Behaviour)
25. Social reward (reward and Threat)
26. Review outcome goal (Goals and Planning)

27. Information about Antecedents (Shaping Knowledge)
28. Reduce Negative Emotions (regulation)
29. Framing/Reframing (Identity)
30. Verbal Persuasion of Capability (Self-Belief)
31. Focus on past success (self-belief)

Appendix XI Procedure for Rapid Scoping Review of barriers and facilitators to appropriate Formula Feeding Behaviour

Studies with a primary qualitative component, published between 2012-2022, and published in the UK or other high-income countries were included to ensure a similar contextual setting as the UK, in which BRIGHT will be delivered. Studies focusing on complementary or exclusive breastfeeding were excluded. The search strategy included a combination of key terms and MeSH terms including but not restricted to infant feeding, guidelines, formula milk'. Searches were undertaken in in six databases (PubMed, CINAHL, EMBASE, Cochrane, Web of Science and Google Scholar). Google Scholar was searched with the addition of 'NOT allergy' due to the large number of irrelevant results regarding cow's milk allergy generated. Finally, hand searching of reference section from included papers from the database search was used to identify any further relevant studies. After screening for inclusion (see Figure S1 for PRISMA flow chart of included studies) 9 records were eligible for inclusion. Relevant to the identified barriers and facilitators will be extracted from each of these studies and synthesised into key themes using Thematic Analysis.

Figure S1. PRISMA Flow Chart of Included Studies in Rapid Review



Appendix XII: Procedure for Development of the BRIGHT Videos

During the initial scoping review and planning phase of BRIGHT, it was deemed important that BRIGHT offer not only written content but also videos to engage caregivers, demonstrate the target behaviours, and align with the existing Baby Buddy approach and design. Five video scripts were initially drafted by KT to cover the topics; i) formula feeding – preparing a bottle and responsive bottle feeding, ii) growth monitoring – understanding growth charts iii) introductory video to bright and iv) complementary feeding – how to introduce solid foods and responsive complementary feeding practices. These video scripts were refined and amended based on PPIE feedback – as described by the procedures outlined in section 6.4.2.2 of the thesis. Moreover, each of these video scripts was reviewed by not only the research team, but also the Best Beginnings content producer as well as their editorial board of experts. Following this process, 2 families were recruited to be filmed for the formula feeding and complementary feeding videos, respectively. The formula feeding family had an 8-week-old formula fed infant, whilst the complementary feeding family had a 2-month old infant. Moreover, key research team members were filmed for the introductory video, growth monitoring video and elements of the feeding videos. Filming and editing were undertaken by an expert filmmaker, with years of experience producing films with families. The filmmaker consulted the research team where questions or issues arose when editing the films. Moreover, the participating families were given a rough cut of the films once available to review and input on. Throughout this process it became clear that 4 videos would each be too long for parents of young infants and for an app-based delivery (~10 minutes each) hence we decided to produce a greater number of shorter videos with a more concise purpose. In total, 9 videos would be included in the BRIGHT prototype; I) BRIGHT welcome film ii) Preparing a Feed iii) Responsive Bottle-Feeding iv) What are growth charts? V) How often should I weight my baby? Vi) How do I use the growth charts in Baby Buddy? Vii) Complementary Feeding viii) Encouraging Healthy Choices ix) Trying New Foods. These videos will be tested in further evaluation of the BRIGHT intervention and thereafter released to the public.

Appendix XIII: Procedure for BRIGHT Theory of Change Model

As a first step, we sought to define the behavioural targets which the BRIGHT intervention seeks to address. A key stage in this process is to consult previous literature to better understand the determinants which may influence those behavioural targets. For BRIGHT, a highly relevant systematic review by Redsell and colleagues was identified. This review highlighted 9 barriers and facilitators to responsive feeding under the Capability component, 5 barriers and facilitators under the Opportunity component, and 4 barriers and facilitators under the Motivation component of COM-B. Moreover, we also included and drew on the barriers and facilitators to appropriate formula feeding behaviours identified under the rapid review in section 6.4.1.2 of the thesis. The second step of the BCW process involves the identification of intervention options and functions that may be best placed to address the barriers and facilitators to behaviour change. Each component of the BCW COM-B Model three functions, which are tools designers can use to address and promote capability, opportunity, and motivation towards performing a desired behaviour. In total 9 functions can be used in tandem to; educate, persuade, incentivise, coerce, train, restrict, restructure the environment, model and enable behaviour change. For the current theory model, the barriers and enablers to responsive milk feeding identified through the Redsell review were mapped onto the COM-B components and functions. The last step of the BCW theory model approach identifies more specific active ingredients of interventions which may be effective in achieving that function called Behaviour Change Techniques (BCTs). The BCT taxonomy of 93 technique was used to identifying relevant BCTs that could be used in the BRIGHT content to promote capability, opportunity, and motivation to responsive feeding. Finally, we added two steps to the approach presented by the BCW above, to produce a complete logical model of how the names BCTs could help to achieve the long-term outcomes of the intervention. Hence, we specified the intended long-term outcomes of the BCTs and intervention components alongside potentially mediating variables. Following the theory of change model exercise, key gaps and opportunities were discussed amongst the team.

Appendix XIV: *PPIE documents with prompts (links):* Formula Round One; Growth; Formula Round Two

Appendix XV: *Tabulation of PPIE Formula feeding and Growth Feedback* [LINKED (password: RAPIDTAB)]

Appendix XVI: Link to Full BRIGHT Prototype for Formula Feeding Resources [LINK]

Appendix XVII: In The Wild Interview Guide

BRIGHT formula feeding, Growth, Sleep, and Crying Prototype "In the Wild" Interview Script Introduction & Fine Print *Greet the participant, thank them for their participation, a little small talk* *Discuss the necessary fine print: So before we get started I wanted to go over a few things with you and give you a little more context. We are creating **some new information and guidance about formula feeding, growth, sleep, and crying for the Baby Buddy app**, which is a free parenting app that's endorsed by the NHS. **Before this new content goes into Baby Buddy we want to test it with parents** who are currently formula feeding young babies, like you, to make sure it's as useful as it can be. **That's why we gave you the prototype for these past two weeks.** This interview is to gather your **feedback** about the experience. We're really keen on getting your **honest** feedback- so please share your thoughts with us- **no one will be offended!** The interview will be structured around a number of questions that we've drafted, and if we have some extra time at the end, please feel free to touch on anything we didn't discuss.

We have scheduled around 60 minutes for today's call, but **I wanted to remind you that you can decide to stop or reschedule this interview at any time during our session. Do you have any questions?**
Great, so let's dive into the questions we have for you about your experience using the prototype.
Quickly, before we do, **can I have your consent to record the interview?** We will **download a transcript** from the interview, which will be **anonymised** (your name will be removed from it), and we will **delete the video recording** to protect your privacy. No one outside of the BRIGHT team will have access to the recording.

begin recording

<u>WHEN: Using the prototype</u>
It'll be great to hear your experience of reading the new content over the last couple of weeks. When I say "content", I mean any information in the prototype you were given.
When did you last take a look at the content? Which parts did you explore then?
How about when you first looked at the content- when was that, and what was your first impression?
When you first accessed the content did you feel you knew where to click?
When else did you access the content, and in what setting?

Prompt: in front of the TV, right before feeding your baby, while cooking, while commuting, with your partner?

<u>WHY: Using the prototype</u>
How/for what purposes did you use the prototype?
Were you looking for anything specific in the content, and did you find what you were looking for?

Prompt: did you remind yourself to check, or were there moments when you wanted to look up information?

AFFECTIVE EXPERIENCE
Did you feel any differently after reading the content?
Prompt: more confident, worried, anxious?

BEFORE & AFTER CONTENT
How did reading the content affect your experience of feeding your baby?
Did you do anything new or anything different after reading the content? How did that go?
Was there anything that helped you make that change?
Was there anything you *wanted* to do (new/different) after reading the info in this section, but that you were not able to do? *Gently: what stood in the way?*

KEY MESSAGES
What do you think were the key messages of the content? *I'm not searching for a specific, or "right" answer, I'm just curious about what you took away from the content*.

IMPRESSIONS
What did you find useful in the content?
Prompt: content, layout, language, tone
Was there anything that you didn't like?
Prompt: content, layout, language, tone
What, if anything, would you change about the content (why/why not)?
Is there anything that you feel you need more information about, that was not covered in this content?

APEASE

<u>APEASE Affordability</u>

Was there anything in the content that did not feel like practical advice?

> Prompt: Did anything feel like it was not practical due to time constraints, cost, energy?
>
> How realistic do you think it is for parents to devote the time and energy it takes to navigate the prototype?
>
> Did any of the content advice or suggestions seem like they would not be possible for some people due to constraints in time or cost?

<u>APEASE Practicability</u>

Was there anything in the content that you felt like you would need additional help to do or understand?

> If so, which parts, and why?
>
> Was there anything in the content that made you feel like you would need support from your **GP or health visitor** to understand or change?

<u>APEASE Effectiveness</u>

Did you feel more confident about choosing a formula milk after reading this content? why/why not?

Did you feel clearer about how much formula milk to offer your baby after reading the guidance on this?

Would you follow the recommendations in the prototype? Why/why not?

After reading the content, did you feel more confident about making up a bottle of milk up safely? Why/why not?

A lot of the content talks about responsive feeding, and I want to ask you a bit about that. I know there was a lot in there, would you like a refresher about what responsive feeding is?

After reading the content would you want to try and feed your baby responsively? Why/why not?
If so, would you feel you knew how to do this? why/why not?

APEASE Acceptability

How do you feel the content compared to your personal set of parenting values? Was there anything that felt at odds with your parenting philosophy?
How did you feel about receiving the content in the form of an app? Would you have preferred to receive the content in a different format?
Was there anything in the content that came off as judgemental, stigmatising, or patronising?
What did you think of the **tone** of the writing?

APEASE Side Effects

Did you have any concerns or negative feelings after reading the content?

Prompt: did you feel any worry, stigma? Do you feel that the content recommended going to a HV or GP more often than you think is necessary? Did the content make you feel concerned about monitoring baby's weight?

Did any of the advice in the content make you look up other materials or consult family members /friends /peers?

APEASE Equity

Did you feel like the content spoke to you personally and represented you and your baby's situation?
Was there anything that made you feel excluded or like the content did not apply to you personally?

Appendix XVIII: BRIGHT Prototype Modifications From In The Wild Interviews

Formula Feeding

- Choosing a formula
  - Question: is there any guidance about shifting/switching formulas?
  - Add: information about goat milk
  - Change: phrasing about different ingredients to be clearer/more explicit
- Preparing formula
  - Question: Are UV sterilisers okay?
  - Add: why aren't prep machines recommended?
  - Add: more detail about preparing ready made formulas
  - Add: how to deal with the sticky/clumpy formula scoop
  - Change: making formula "quickly" → making formula as quickly/conveniently as possible?
  - Change: emphasise even more strongly not to add extra powder
- How much to give
  - Question: Is there a maximum amount of formula you can give within 24h?
  - Add: formula milk amounts if your baby is premature
  - Add: *How often should I feed?* Article
  - Add: Address the formula per kg vs formula per age guidance
  - Change: Layout of formula table to smaller age bands
- Other
  - Question: Can I mix different kinds of formula?
  - Question: Can I add medicine to formula?
  - Add: *What is formula?* Article
  - Add: more information about giving water
  - Add: information about milk rashes from feeding
  - Add: combination feeding information
  - Add: more information on ***how*** to make time for yourself, not just saying it's important
  - Add: more information about splitting feeding with partner or support system
  - Add: a section on equipment, including an overview on bottles and teats
  - Add: suggestion to add guidance about colic drops
  - Change: suggestion to move top tips further down so people read the full articles
  - Change: suggestion to change "quiz" to a more scientific word

- Change: suggestion to edit article title as follows: *"Overcoming some of the challenges you might face when ~~**responsive**~~ feeding"*

Growth

- Add: link to weighing venues or opportunities per NHS region
- Add: videos from parents who dealt with their child crossing centiles, and what they did in response
- Add: A bit more information about weight dropping after birth
- Add: reminders to get baby weighed at regular appointments

Crying

- Add: it's usually okay if your baby is crying less than you expect, it doesn't necessarily mean anything is wrong

General

- Question: Is it going to be called BRIGHT?
- Add: an introduction acknowledging that everyone is doing their best
- Add: a short article/information on who wrote BRIGHT (for credibility)
- Add: timelines for what to expect throughout the first year (like the stages of sleep) for the other sections
- Add: A search function within BRIGHT
- Add: formula feed tracker and growth centile feedback
- Add: specific missing crosslinks
- Add: FAQ's in the formula feeding section and on the homepage of BRIGHT
- Add: the videos would have been helpful
- Change: visuals in the different sections should be relevant (e.g. Formula feeding section should have a bottle, not an apple)
- Change: Restructure formula feeding articles into sections so it's easier to navigate

Appendix XVIX: Description of Appetite Quizzes and Infant Feeding Tracker

Appetite Quizzes

There is a growing evidence base demonstrating that parental feeding practices can be modified in response to a child's appetite as early as infancy. The challenges and barriers to responsive feeding parents may face when feeding their child may greatly differ based on their child's appetite traits. For instance, guidance on managing crying without feeding or recognising fullness cues may be more relevant to a hungrier, or food responsive infant. In contrast, guidance on not pressuring when bottle feeding and paced feeding may be more relevant to a less hungry infant. Hence, we sought to explore the acceptability and feasibility of delivering feeding guidance tailored to infant appetite through an appetite quiz feature. This quiz would inform parents of their child's appetite profile alongside tailored feeding guidance.

Characterisations of the child's appetite profile was delivered using the Baby Eating Behaviour Questionnaire (BEBQ),. A cut off score was generated to distinguish a 'hungry' appetite from a 'typical' appetite using ROC analyses. Tailored feedback was developed for both the 'hungry appetite' and 'typical appetite' groups and refined in collaboration with PPIE. Following the development of the Baby Appetite Quiz, we developed two further Toddler appetite quizzes using the Child Eating Behaviour Questionnaire Toddler Version (CEBQ-T), one on general toddler appetite and one on fussy eating profiles. The latter used the food fussiness subscale of the BEBQ. Cut off scores could not be reliably generated for the CEBQ-T quizzes; therefore, the median score was used as a cut off. Once again, the tailored written feedback was developed using PPIE (n=6 caregivers) and tested using think-aloud interviews (n=12 caregivers). This confirmed that information about infant appetite is welcomed amongst caregivers and may be effective in motivating appropriate formula and complementary feeding behaviours.

Formula Feeding Tracker

During the initial rounds of PPIE interviews and feedback, caregivers reported an unmet need for a user-friendly and non-commercial formula feeding tracker or diary. Given the emphasis placed on monitoring of formula feeding volumes and increasing self-efficacy to feed within the guidelines within Baby Milk – we decided to develop a protocol for a formula feeding tracker. The tracker will be specifically designed for monitoring infants who are formula fed, and will provide parent users with feedback on their infant's formula milk intake in line with the WHO guidelines over a 24 hour period. Moreover, drawing on BCW's COM-B Model this provides caregivers with the ability to self-monitor their formula feeding behaviours and receive feedback on their infant's formula milk intake – two important BCTs for behaviour change. Overall, the exercise will: focus participants' attention on feeding behaviour; increase participants' awareness of their feeding volumes; and increase participants' awareness of the guidelines. Moreover, where parents are feeding above or below the guidelines they will be pointed to relevant resources, to support a more tailored guidance approach. We will also use the tracker to give parents real time feedback on how their feeding practices align with the recommendations for their infant at the appropriate age. The tracker would also allow us to collect detailed information on formula milk intake to test the efficacy of the intervention in a randomised controlled trial. Hence, parents would be nudged to use the tracker once a month to check in on their feeding, but would be able to use it as much as they like in the Baby Buddy App. Following the development of a detailed proposal with the Best Beginnings team, it was estimated that for the tracker to be developed in line with the intended protocol, we would require an extra ~£60,000 of funding. As these funds we're not available to us, we will apply for funds to co-develop a formula feeding tracker, using the PBA, within the next grant application.

Appendix XX: Growth Feedback Messages

Introductory Article: Feedback on your baby's growth

Sometimes it can be reassuring to have a bit of feedback and individual guidance about your baby's growth. Please look back on your baby's last weight measurement in the red book, and find your baby's weight centile (what's a weight centile? click here to find out!).

Once you've found your babies centile group, please select your baby's category below.

=<2nd centile

Your baby's weight is in the very low centile range. This may be healthy for some babies, if the parents are short (Dad is 5 ft 4 in (163 cm) or less, or Mum is at 4 ft 11 in (150 cm) or less). Otherwise, it might mean that your baby is not drinking as much formula milk as they need.

You can find out more about how much formula a baby needs (click here) and responsive feeding (click here).

All children grow at their own rate, and they don't always follow a centile line. But if your baby's weight has gone down by crossing a whole centile space, that means they are not gaining weight as fast as expected. It's a good idea to talk to your health visitor.

To find out more about baby weight centiles, watch the video here.

=<9th centile

Your baby's weight is in the low centile range. That's normally healthy if they were born in the low centile range or if the parents are short (Dad is 5 ft 6 in (168 cm) or less, or Mum is at 5 ft 1 in (155 cm) or less). If the parents aren't short, it might mean that your baby is not drinking as much formula milk as he needs.

You can find out more about how much formula a baby needs (click here) and responsive feeding (click here).

All children grow at their own rate, and they don't always follow a centile line. But if your baby's weight has gone down by crossing a whole centile space, that means they are not gaining weight as fast as expected. It's a good idea to talk to your health visitor.

To find out more about baby weight centiles, watch the video here.

9th-75th centile

Your baby's weight centile is in the medium range. That's normally healthy.

All children grow at their own rate, and they don't always follow a centile line. But if your baby's weight has gone up by crossing a whole centile space, that means they are gaining weight quickly. They may be drinking more formula milk than they need. You can find out more about how much formula a baby needs (click here) and responsive feeding (click here).

And if your baby's weight has gone down by crossing two centile spaces, that means they are not gaining weight as fast as expected. It's a good idea to talk to your health visitor.

To find out more about baby weight centiles, watch the video here.

75th-91st centile

Your baby's weight centile is in the moderately high range. That's normally healthy.

All children grow at their own rate, and they don't always follow a centile line. But if your baby's weight has gone up by crossing a whole centile space, that means they are gaining weight quickly. They may be drinking more formula milk than they need. You can find out more about how much formula a baby needs (click here) and responsive feeding (click here).

And if your baby's weight has gone down by crossing two centile spaces, that means they are not gaining weight as fast as expected. It's a good idea to talk to your health visitor.

To find out more about baby weight centiles, watch the video here.

=>91st centile

Your baby's weight is in the high centile range. That’s normally healthy if they were born in the high centile range or if the parents are tall (Dad is at least 6 ft 1 in (185 cm), or Mum is at least 5 ft 8 in (172 cm). If the parents aren’t tall, it might mean that your baby is drinking more formula milk than they need.

All children grow at their own rate, and they don’t always follow a centile line. But if your baby's weight has gone up by crossing a whole centile space, that may mean that they are gaining weight quickly. They may be drinking more formula milk than they need.

You can find out more about how much formula a baby needs (click here) and responsive feeding (click here).

To find out more about baby weight centiles, watch the video here.

Appendix XXI Full Theory of Change Table [LINK] and Description

Description of Theory of Change

Firstly, the Redsell review highlighted how a baby's individual appetite for feeding can be a key barrier to responsive feeding from birth. For example, it may be more difficult to practice responsive feeding with infants of a higher appetite – where offering milk in response to expressed hunger cues could lead to overfeeding. Similarly, where an infant expressed a lower appetite – parents may also be more likely to perform pressured feeding behaviours such as encouraging them to finish a bottle. In order to address this barrier to responsive feeding, and thereby achieving the target outcome of reducing RIWG, BRIGHT tools must help parents to feed responsively in the context of their child's appetite. Hence, by developing a prototype for the appetite quiz – which seeks to identify and offer tailored tips for responding to an infant appetite - we sought to support parents' *capability* to recognise their child's appetite profile. Moreover, we sought to encourage their *motivation* to feed responsively in the context of their child's appetite using numerous BCTs beyond education. These included; feedback on behaviour, framing/reframing, and verbal persuasion about capability. Secondly, the Redsell review pointed to conflicting feeding guidance and advice as a barrier to responsive feeding. Similarly, the rapid review pointed to missing and mixed messaging within feeding guidance as a barrier to appropriate feeding. As BRIGHT is largely an education based intervention which seeks to provide a comprehensive library of support for formula feeding – this barrier is addressed throughout the intervention. For example, we created 'myth busting' FAQs resources that would address common confusions and misinformation on formula feeding which emerged from PPIE feedback. This is just one example of how BRIGHT seeks to promote caregivers' *capability* to feed responsively. Moreover, within BRIGHT– numerous BCTs outside of education are utilised to encourage caregiver's self-efficacy. These include the BCTs of verbal persuasion about capability and identification of self as a role model. Moreover, to promote to target the *motivation* component of COM-B and the facilitator to responsive feeding 'feeding goals, intentions and plans' - we included a formula feeding tracker in the BRIGHT prototype. This sought to incorporate the BCTs; action planning, habit reversal, prompts/cues, self-monitoring of behaviour, and goal setting. Finally, a key barrier that was thoroughly addressed in BRIGHT was the low accessibility of feeding guidance. Given its integration into the Baby Buddy app, BRIGHT is developed to be of a reading of age ~12. Moreover, caregivers of diverse backgrounds provided input into the BRIGHT resources to ensure they would be practical, accessible, and understandable to parents. For instance, this led to the development of detailed resources on growth charts and infant growth patterning – to address the common confusion amongst parents on this topic.

Given BRIGHTs format as an app-based education intervention that is delivered at the individual level, there were a few barriers to responsive feeding we were largely unable to address. First, we were not able to alter the structural/environmental factors or the influence of the social environment. However, we did make substantial effort to maximize the accessibility of guidance provided by BRIGHT to those living under greater deprivation and avoid exacerbating structural inequalities. Similarly, we could not fully address the barrier 'social and cultural norms and expectations' around responsive and formula feeding. However, we did seek to reduce misperceptions around infant feeding and reduce internalized stigma and negative emotions surrounding formula feeding which were reported by parents throughout the PPIE process. To do so, we included written and video resources that sought to 'reduce negative emotions' and 'frame/reframe' formula feeding stigma by presenting guidance in a positive and supportive tone and addressing the common stigma's felt by parents.