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The aetiology of emotional eating in childhood and its association with weight

Transfer from MPhil to PhD

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Contents

Abstract

Overeating in response to negative emotion and stress (so-called 'emotional overeating', EOE) has been of great interest in relation to obesity risk. Experimental studies with animals and humans suggest that, for some individuals, negative emotions and stress can result in increased intake of palatable food. This tendency may therefore predispose these individuals to overweight. The idea that the tendency to eat less in response to negative emotions (so-called 'emotional undereating', EUE) may be protective against obesity has received considerably less attention. In addition, the relationship between EOE and EUE has not been established. In particular, it is not known if EOE and EUE reflect different aspects of the same underlying trait (the tendency to either over- or under-eat in response to negative emotion), or are distinct behaviours.

Little is known about the aetiology of emotional eating, especially in childhood. The Psychosomatic Theory of Obesity suggests that some individuals learn how to regulate their emotions using food during childhood, predisposing those individuals to obesity. There is some evidence that emotional eating does indeed develop early, and tracks into later childhood. Understanding its aetiology in early life and its impact on weight would help to clarify the role of this behaviour in childhood obesity risk.

Twin studies provide a powerful method for understanding the extent to which individual differences in a characteristic are determined by genetic or environmental variation. This thesis uses data from Gemini, a large population-based cohort (n=2402 families) of British twins to: (i) quantify for the first time the relative contribution of genes and environment to the development of emotional over- and under-eating in early life; (iii) explore for the first time the common aetiology underlying EOE and EUE; (iii) identify modifiable environmental influences that shape the development of EOE and EUE in childhood; (iv) establish the relationship between emotional over- and undereating and weight and (v) investigate how genetic and environmental factors interact to contribute to the risk of emotional eating.

1 Obesity

1.1 Prevalence and health implications

Obesity continues to be one of the greatest health challenges of our time. Over the past 30 years, rates of obesity have been rising from 29% to 37% in men and from 30% to 38% in women worldwide (Ng, Fleming et al. 2014). Obesity rates in developing countries are 'catching up' with developed countries, contributing to the growing burden (Swinburn, Sacks et al. 2011; Jones-Smith, Gordon-Larsen et al. 2012). A similar increase has been observed in children as well, with more than 20% of boys and girls (aged 2-19 years) classified as obese in 2013 (Ng, Fleming et al. 2014). Recent research suggests that although rates of child and adult obesity in the developed world are plateauing (Olds, Maher et al. 2011; Sperrin, Marshall et al. 2014), the prevalence will remain high. Simulation modelling projects that by 2030 the UK will have 11 million more obese individuals, resulting in a rise of obesity-related health problems such as diabetes, heart disease and cancer (Wang, McPherson et al. 2011). Next to physical health problems, psychological wellbeing is also affected. Tracking a representative sample from early adolescence into late adolescence revealed that overweight and obese children were at higher risk of developing mental health problems such as depression and attention/hyperactivity deficit disorder; and overweight and obese children did poorer at school and missed more days of schooling compared to their normal weight peers (Duarte, Sourander et al. 2010; Halfon, Larson et al. 2013).

1.2 Childhood obesity and weight tracking

The increase in childhood obesity is especially alarming. Overweight and obese children tend to continue to have weight problems across their lifetime (Singh, Mulder et al. 2008) increasing their risk of disease and shortened lifespan (Baker, Olsen et al. 2007; Reilly and Kelly 2011; Park, Falconer et al. 2012). A study that followed children from birth into mid-adolescence, found that the strongest predictor of adolescent weight was childhood weight, unrelated to weight at birth or familial cases of obesity (Fuentes, Notkola et al. 2003). A second study found that childhood BMI at 12 years was predictive of adult BMI at 35 years (Trudeau, Shephard et al. 2003). Additionally, it has been reported that almost half of children in the highest BMI quintile remain there in adulthood, highlighting that childhood obesity persists into later life (Herman, Craig et al. 2009), and underlines the importance of understanding the drivers of childhood obesity.

1.3 Genetic and environmental influences on weight in early life

In order to tackle the obesity epidemic, research has aimed to understand the aetiology of obesity and the causal factors involved in excess weight gain in childhood. Identifying ways to prevent childhood obesity is key to reducing the current rates of obesity.

1.3.1 Twin studies

1.3.1.1 Key assumptions of the twin method

Twin studies are an invaluable methodology for investigating the relative contribution of genetic and environmental influences on variation in complex human traits. Twin research exploits the natural occurrence of identical (monozygotic, MZ) and non-identical twins (dizygotic, DZ). Identical twins are natural genetic clones of one another, sharing 100% of their genome; whereas non-identical twins share on average 50% of their segregating genes, in keeping with regular siblings. Importantly, both types of twins share their environments to a very similar extent insofar as they are gestated in the same mother at the same time, are exactly the same age, and grow up in the same family. This means that resemblance between MZ and DZ twins can be compared to estimate genetic and environmental contributions to any given trait. If MZ pairs are more similar than DZs, we assume that genetic factors must be contributing to this difference, because the only real difference between the two types of twins is that MZs are twice as similar genetically (because the extent to which environmental factors are shared is equal for both types of twins). As 'a rule of thumb', genetic influences can be estimated broadly by doubling the difference between the MZ and DZ correlations. The statistic derived is heritability, which quantifies the proportion of trait variation attributable to genetic variation, and can be thought of as an index of the genetic effect size ranging from 0% (genes do not contribute at all to trait variation) to 100% (genes entirely explain trait variation). Environmental effects are also estimated, and separated out into those that are completely shared between siblings (those factors that contribute to their similarity, such as socioeconomic status), and non-shared (those that contribute to sibling differences, such as having different friends, or one twin having a serious illness) (Polderman, Benyamin et al. 2015).

The twin method is based on some key assumptions. In order to extrapolate findings from a twin study to the wider population, twin cohorts must be representative. Importantly as well, the 'equal environments assumption (EEA)' must be met. The EEA is that the environmental factors contributing to variation in the trait are shared by, and effect MZ and DZ twins to the same degree. For example, if MZs are treated more similarly than DZs and this contributes to increased similarity between them

on a particular trait, the EEA has been violated. Furthermore, the twins themselves or the participants (parents, teacher, doctors) rating the behaviour of the twins, must not be influenced by the twins' zygosity. For example, if the twins themselves or other raters assume that they are identical, they might be biased in their responses, perhaps reporting that they are more similar than they actually are, resulting in unreliable estimates (Rijsdijk and Sham 2002). This issue is particularly pertinent in young samples for whom most behavioural data are parent reported. A more detailed account of the twin method and its assumptions and limitations is provided in Chapter 5 of this report.

1.3.1.2 Twin based heritability estimates for adult BMI

In adulthood the heritability of BMI is high. Meta analyses have estimated that 50% to 90% of variation in BMI is explained by genetic effects (Maes, Neale et al. 1997; Elks, den Hoed et al. 2012; Min, Chiu et al. 2013). On the other hand, shared environmental effects do not contribute at all with the remaining variation being attributable to environmental factors unique to each individual (Maes, Neale et al. 1997; Elks, den Hoed et al. 2012; Min, Chiu et al. 2013).

1.3.1.3 Twin based heritability estimates for childhood BMI

Twin Studies focusing on the heritability of weight in childhood have suggested lower estimates of genetic effects in earlier childhood and significant effects of environmental factors shared between the twins (Elks, den Hoed et al. 2012). Longitudinal twin studies tracking BMI from birth to adulthood have confirmed lower heritability estimates in childhood but suggest a constant increase of genetic effects across time. A British study observed that at four years of age 49% of individual differences in BMI were explained by genetic variation, and shared environmental factors were also substantial (36%). Strikingly, by age 7, estimates were found to have changed considerably with genetic effects increasing to 77%, whereas shared environmental effects dropped to 6% (Haworth, Carnell et al. 2008). Meta-analyses have concluded that the heritability of BMI continues to rise throughout adolescence, reaching its peak at about 20 years of age (about 80%) after which it plateaus over adulthood (Elks, den Hoed et al. 2012). More recently, outcomes of the CODAT (Collaborative project of Development of Anthropometrical measures in Twins), an international collaboration of twin studies including more than 87000 pairs of twins, confirmed previous analyses that shared environmental factors explain variation in BMI only in childhood and early adolescence. Genetic effects were found to be lowest at around age four (41%) and then increase steadily (Silventoinen, Jelenkovic et al. 2016), while shared environmental factors are highest around this age (40%), and subsequently diminish over the course of adolescence.

In summary, adult twin studies propose that individual differences in BMI are mainly explained by genetic variation; and any environmental factors involved are specific to the individual (not those that are shared between two twins in a pair). However, in young children genetic factors are less important than in adulthood, and there are important influences of the shared environment; but these diminish with increasing age, replaced by increasing genetic effects.

1.3.2 Genome-wide association studies

Twin studies estimate the relative contribution of genetic and environmental effects very broadly, but they do not provide information about which specific genes or environmental factors influence BMI. Genome-wide Association Studies (GWAS) can be used to identify specific genetic variants associated with BMI. Using contemporary genome wide sequencing technology participants' genomes are coded. Genome wide sequencing chips map millions of single nucleotide polymorphisms (SNP) scattered across the genome. SNPs are loci in the genome where individuals differ at the level of one singular nucleotide base pair (i.e. a single letter change in the genetic code). Variation in each SNP is then correlated with BMI, one by one for all of the available SNPs. Sometimes this results in upwards of a million statistical tests (if there are a million available SNPs), and the p-value is therefore adjusted accordingly to account for such a large number of tests. The current convention for GWAS is to set the alpha level at $p < .5 \times 10^{-8}$ (Dudbridge and Gusnanto 2008). This methodology enables the identification of specific locations in the human genome that contribute to variation in BMI. However, it has in fact proved very difficult to identify many obesity-associated SNPs through GWAS. This has been partly due to the small effect sizes of each SNP on BMI (the largest effect size of any SNP identified to date explains approximately 0.5% of variation in BMI), and partly because of the very stringent significance threshold to account for the many tests performed. The combination of small effects and stringent adjustment has meant that very large sample sizes are needed to detect significant relationships.

The most recent meta-analysis of obesity-related SNPs reported that 97 SNPs have been identified that are robustly associated with BMI (Locke, Kahali et al. 2015). The most important of these was the 'fat mass and obesity associated gene', FTO. Located on chromosome 16, FTO has been consistently associated with BMI (Scuteri, Sanna et al. 2010). Adults of average height who carry two copies of the high risk version (AA) are on average three kilograms heavier than those who carry two copies of the low risk version (TT), and heterozygotes (one low risk and one high risk version) are approximately 1.5 kilograms heavier than the TTs (Frayling, Timpson et al. 2007). Gene expression studies suggest that

FTO is part of the regulatory system of energy uptake in the hypothalamus (Olszewski, Fredriksson et al. 2009), a key brain area involved in the central control of appetite (King 2006; Meister 2007). There is now considerable evidence that FTO influences weight via an appetitive pathway. Food deprived rats were found to have excess FTO expression in their hypothalamus (Fredriksson, Hagglund et al. 2008), and children with two copies of the high risk version are less sensitive to satiety (fullness) than those who carry a low risk version of the variant (Haworth, Carnell et al. 2008). More recently, research has shed some more light on the biological mechanisms through which FTO influences appetite. Following food intake, carriers of the high risk FTO allele reported higher levels of hunger, a dysregulation of the 'hunger hormone' ghrelin and different activation patterns of reward and appetite associated brain areas. These results suggest that FTO is modifies appetite centrally (Karra, O'Daly et al. 2013).

Although the field was initially excited by the discovery of FTO, subsequent discoveries were somewhat disappointing. Even in the aggregate, the 97 SNPs identified to date only account for 2.7% of the variance in BMI, highlighting the small effect of each individual SNP. These findings support the idea that many genes are involved in the determination of human body weight (so-called 'polygenic'), each contributing a very small effect (Locke, Kahali et al. 2015). Importantly, however, many of the other SNPs also appear to be influencing weight via effects on appetite. Research combining the effects of many of the 97 SNPs (by creating an aggregate genetic risk score) has shown that genetic risk of obesity is associated with reduced satiety sensitivity, even when FTO is excluded from the score (Llewellyn, Trzaskowski et al. 2014), as well as other eating behaviours including uncontrolled eating and emotional eating (Konttinen, Llewellyn et al. 2015).

In the light of GWAS findings, researchers have concluded that it is not yet possible to predict obesity risk using genetic markers with any precision. However with increasing sample sizes more and more genetic risk markers are likely to be detected, and this may become a feasible endeavour in the future. Nevertheless, together with twin studies molecular genetic methods provide evidence for the genetic influence on obesity; but it is clear that BMI is a complex human trait, influenced by a multitude of genetic and environmental factors (Morandi, Meyre et al. 2012; Waalen 2014) that appear to vary during different developmental phases across the lifespan.

1.3.3 The 'obesogenic' environment

Twin and molecular genetic studies have demonstrated the significant influence of genetic factors on obesity. However, while genetic factors might partially explain individual differences in weight, they cannot account for the recent rapid rise in obesity rates in both children and adults. The human genome is changing very slowly through evolutionary selection and pressure, and has been relatively stable throughout human history. In comparison, changes to our food and activity environments have occurred rapidly and are widely believed to have caused the dramatic increases in population weight over the last 50 years. For example, portion sizes for many foods (both in terms of foods sold as well as consumed) have increased (Piernas and Popkin 2011), and experimental work has demonstrated that increasing the portion size of food on offer results in overconsumption. For example, during a 1-week experimental study with a cross-over design, adult participants presented with both larger and smaller portion sizes consumed one third more energy in the larger portion condition; and they gained weight (Rolls, Morris et al. 2002). Comparing portion sizes over the past three decades in the US, research has shown that portion sizes have grown significantly (Piernas and Popkin 2011), possibly resulting in increased energy intake at every sitting. A previous meta-analysis suggested that doubling the portion size leads to an increase of food intake of 35% (Piernas and Popkin 2011; Zlatevska, Dubelaar et al. 2014). Additionally easily available energy dense snack foods (Farley, Baker et al. 2010) as well as the low cost of a diet consisting mainly of processed energy dense food (Rao, Afshin et al. 2013), have the potential to contribute to overconsumption and increased calorie intake.

Accompanying this population-level rise in energy intake is a progressively more sedentary lifestyle with lower energy expenditure. Changes to the built environment and sedentary leisure activities in the home (such as increased television viewing) have been shown to result in a more inactive lifestyle (Brownson, Boehmer et al. 2005). A 2012 study found one third of the adult population and the majority of teenagers (80%) in the UK did not meet the minimum requirement of one hour of moderate exercise per day (Hallal, Andersen et al. 2012). This low rate of physical activity has been suggested to be one of the major contributors to population weight gain and obesity (Ness, Leary et al. 2007). Increasing physical activity is therefore often one of the proposed strategies for obesity prevention and management. However, the relationship between physical activity and obesity is complicated. A recent review concluded that physical activity has little effect on weight loss, but might be useful for weight maintenance, and as a strategy to prevent further weight gain (Malhotra, Noakes et al. 2015). In addition, one study investigated the causal link between physical activity and child obesity using Mendelian Randomisation. This method that takes advantage of genetic risk of disease

to index the disease itself without environmental confounding, enabling cause-effect relationships to be established (Lawlor, Harbord et al. 2008). Results suggested that having a high BMI reduces the amount of physical activity a child participates in, challenging the argument that low physical activity leads to higher BMI (Richmond, Smith et al. 2014). Together, recent changes in diet, lifestyle, and food availability have created what researchers have termed the current 'obesogenic environment', an environment that promotes the development of obesity.

1.3.4 Gene-environment interactions in obesity

To further understand the aetiology of obesity, research has aimed to understand how genetic and environmental factors might interact. Gene-environment interaction implies that the genetic influence on any given trait (in this case, BMI) is moderated by the presence of certain environmental conditions – i.e. certain environmental conditions will amplify the genetic influence on BMI, other exposures will buffer it. There is convincing evidence that physical activity might protect individuals at higher genetic risk of obesity, by attenuating the genetic effect on BMI. For example, a twin study showed that the heritability of BMI is lower in active compared to non-active male adults (Mustelin, Silventoinen et al. 2009). Additionally the association between genetic risk of obesity (indexed from established obesity-associated SNPs) and BMI was found to be smaller in active compared with inactive adults (Li, Zhao et al. 2010; Kilpelainen, Qi et al. 2011). Compared to the active participants, those who were physically inactive had an increased weight per genetic obesity risk loci (592 grams per genetic obesity risk loci, versus 379 grams) (Li, Zhao et al. 2010). Research has also examined the relationship between genetic risk for obesity and dietary factors. The difference in weight between adults at high versus low genetic risk for obesity (indexed using an aggregate score of obesity-associated SNPs) was greater for those individuals who consumed high amounts of sugar-sweetened beverages (Qi, Chu et al. 2012). These findings propose that genetic and environmental factors of obesity interact and highlight the complex aetiology underlying the development of weight problems.

1.4 Behavioural susceptibility model of obesity

A wealth of twin research has shown that despite the recent obesity epidemic, individual differences in BMI continue to be driven largely by genetic variation. Recent research employing genotyping technology has been able to identify specific genetic variants (in the form of SNPs) associated with obesity risk. At the same time, changes in our environment have been identified as important drivers of the current obesity epidemic. This has created a seeming paradox of both genetic and environmental determination of weight that has been difficult for researchers to explain. The Behavioural Susceptibility Model was developed in order to make sense of the problem of dual determination of weight by both genes and the environment.

An important observation is that despite the increasingly obesogenic environment not everyone is overweight. The obesogenic environment seems to affect heavier individuals disproportionately resulting in morbid obesity rates increasing twice as fast as rates of mild obesity (Wardle and Boniface 2008; Llewellyn and Wardle 2015). This suggests that those individuals at highest genetic risk have gained the most weight, implying gene-environment interaction in modern obesity. In support of this, comparisons of twin studies investigating adult BMI have shown how genetic effects have increased steadily over the past few decades. A large study of Swedish siblings and twins born between 1951 and 1983 showed that genetic variance in BMI was higher for those born later (and by implication living in a more 'obesogenic' food environment) (Rokholm, Silventoinen et al. 2011). These findings have been further substantiated by molecular genetic studies; the effects of FTO (Rosenquist, Lehrer et al. 2015) and of a genetic risk score (Demerath, Choh et al. 2013) on BMI are significantly stronger for those born later, suggesting that genetic effects are moderated by environmental exposure. These findings support the notion that the modern obesogenic environment is eliciting genetic effects on weight, and that individuals at higher genetic risk of obesity are affected more by environmental drivers of it (Wardle and Boniface 2008; Rokholm, Silventoinen et al. 2011).

In order to explain how both genes and the environment contribute to obesity risk, a Behavioural Susceptibility Model has been proposed. The Behavioural Susceptibility Model proposes that appetitive processes (expressed as distinct eating behaviours) sit on the causal genetic pathway – i.e. 'obesity genes' are influencing weight through their effects on appetite. In particular, appetitive traits such as responsiveness to food cues (wanting to eat when you see, smell or taste delicious food), and sensitivity to internal satiety (how long you take to feel full once you start eating, and how long you remain full for before you feel hungry again) have been proposed as mediators between genetic risk

for obesity and the obesogenic environment (Carnell and Wardle 2008; Llewellyn and Wardle 2015). Individuals who are more food responsive and less satiety sensitive, by virtue of their genetic endowment, are more likely to overeat in response to the current food environment and to become obese.

The Child Eating Behaviour Questionnaire [CEBQ] (Wardle, Guthrie et al. 2001) is a widely used parent report psychometric measure of eight weight-related eating behaviours in childhood: Satiety Responsiveness (SR), Food Responsiveness (FR), Enjoyment of Food (EF), Slowness of Eating (SE), Food Fussiness (FF), Desire to Drink (DD), Emotional Overeating (EOE), and Emotional Undereating (EUE) (Wardle, Guthrie et al. 2001; Carnell and Wardle 2007). It was developed in order to test the key assumptions of the Behavioural Susceptibility Model of weight: (i) appetitive traits are causally associated with weight, and (ii) appetitive traits have a genetic basis. A wealth of data have provided evidence that children with more avid appetites (higher FR, EF, DD and EOE; lower SR, SE, FF and EUE) are heavier (Webber, Hill et al. 2009; van Jaarsveld, Llewellyn et al. 2011; Quah, Chan et al. 2015; Steinsbekk and Wichstrom 2015). The heritability of SR and EF were also established in 10-year old British twin children, with strikingly high estimates for both traits (63% and 75% respectively) (Carnell, Haworth et al. 2008; Llewellyn, van Jaarsveld et al. 2010). The CEBQ has therefore contributed evidence to support the idea that there are a range of appetitive traits that both predispose to higher weight, and have a genetic basis. Importantly, the stability of these appetitive traits from four to 11 years of age was explored, with fairly modest prospective associations observed, indicating that these traits are already well established by early childhood (Ashcroft, Semmler et al. 2008).

The Baby Eating Behaviour Questionnaire (BEBQ) was developed in order to explore the genetic basis of these traits in infancy, to establish when genes start to influence appetitive traits (Llewellyn et al, 2010), and whether they play a role in infant weight gain. The BEBQ is a modified version of the CEBQ, and measures four of the traits (SR, SE, FR and EF) during the period of exclusive milk-feeding, before any solid food has been introduced. Prospective data using the BEBQ have indicated that these appetitive traits appear to play an important causal role in early infant weight gain (van Jaarsveld, Llewellyn et al. 2011; van Jaarsveld, Boniface et al. 2014). In addition, the heritability of all of these appetitive traits have also been established for the infancy period and were found to be high even in this early developmental phase (72%, 84%, 59%, 53% respectively). These data provide some support for the Behavioural Susceptibility Model of obesity, insofar as variation in some of these appetitive traits are present from early life, appear to drive early weight gain, and have a strong genetic basis.

However, the role of emotional eating (both over- and under-eating) as a behavioural mediator of genetic risk of obesity is largely unknown. It has never been studied in early childhood – neither its relationship with weight, nor its genetic basis have been established, and it is unclear if this behaviour tracks over time in line with some of the other appetitive traits (Ashcroft, Semmler et al. 2008).

2 Emotional eating in childhood

Emotional eating is a key eating behaviour linked to the development of adult obesity (Macht 2008), although it has been much less studied in children. Emotional eating describes the tendency to regulate emotions and stress with increased food intake ('emotional overeating', EOE), or to experience downregulation of appetite in response to negative emotions ('emotional undereating', EUE). The tendency to overeat in response to negative emotions and stress has been reported to start early in life. A study exploring the continuity of eating behaviours (longitudinal tracking) measured by the CEBQ from four to 10 years found substantial tracking for EOE (r=0.45) and moderate tracking for EUE (r=0.29). Other noteworthy observations were that the mean for EOE increased significantly across time (from 1.8 to 2.1), indicating that in general children engaged slightly more in this behaviour as they got older. Contrastingly, the mean for EUE decreased significantly (from 2.9 to 2.7), indicating that children were slightly less likely to demonstrate this behaviour as they got older (Ashcroft, Semmler et al. 2008).

2.1 Emotional eating and childhood weight

Previous research examining the effect of emotional eating on childhood weight has included mostly cross-sectional studies, and results have been mixed. A full list of the studies investigating the association between emotional overeating and emotional under-eating and weight in childhood is provided in Table 2.1 and Table 2.2.

The studies conducted have used different measures to assess emotional eating behaviour. The Dutch Eating Behaviour (DEBQ) was used by nine studies (Wardle, Marsland et al. 1992; Braet and Van Strien 1997; Caccialanza, Nicholls et al. 2004; Braet, Claus et al. 2008; Jahnke and Warschburger 2008; van Strien and Oosterveld 2008; Jollie-Trottier, Holm et al. 2009; Snoek, Engels et al. 2013). The DEBQ (Vanstrien, Frijters et al. 1986) aims to measure three key eating behaviours in adults: external, restrained and emotional eating. To investigate eating behaviours in children, the Dutch Eating Behaviour Questionnaire for Children (the DEBQ-C) was developed (van Strien and Oosterveld 2008). In addition to this, a parented report version of the DEBQ (the DEBQ-P) has been designed (Braet and Van Strien 1997) to allow these eating behaviours to be measured in toddlers and children who are too young to respond for themselves. In comparison to the Child Eating Behaviour Questionnaire (CEBQ) (Wardle, Guthrie et al. 2001) the DEBQ does not include items relating to emotional under-eating.

Most research investigating the association between emotional over- and under-eating, and weight in samples of children has used the CEBQ (Viana, Sinde et al. 2008; Joyce and Zimmer-Gembeck 2009; Webber, Hill et al. 2009; Parkinson, Drewett et al. 2010; Spence, Carson et al. 2011; Svensson, Lundborg et al. 2011; Cao, Svensson et al. 2012; Jansen, Roza et al. 2012; Silva, Capurro et al. 2013; Braden, Rhee et al. 2014; dos Passos, Gigante et al. 2015; McCarthy, Chaoimh et al. 2015; Steinsbekk and Wichstrom 2015), which includes items on both emotional over and under-eating.

One study (Hajna, LeBlanc et al. 2014) used the Family Activity Eating Habit Questionnaire to assess emotional eating behaviour. The Family Activity Eating Habit Questionnaire (FAEHQ) is a family based questionnaire which includes parent rated items regarding emotional overeating, similar to the CEBQ and DEBQ, such as "How often does your child eat when angry or in other negative mood states" (Golan and Weizman 1998). A table listing all of the questionnaires and their items tapping into EOE and EUE can be found in the Appendices 1 and 2.

Studies have also differed in their adiposity measures. Most studies used height and weight data to calculate the BMI of the participants. Studies using BMI have tended to report BMI standard deviation scores (BMI-SDS) using population reference data, taking into account the age and sex of each child. Specifically, each BMI score is converted to a BMI-SDS relative to the mean of the reference data, for their exact age and their sex (BMI-SDS < 0 are lower than the mean; BMI-SDS > 0 are above the mean; BMI-SDS equal to 0 are the same as the mean for the reference data). Some studies have used the BMI scores to classify participants as normal weight, overweight and obese using different guidelines, such as the Centres for Disease Control and Prevention (CDC) and the International Obesity Task Force (IOTF). One study used multiple measures of adiposity (Hajna, LeBlanc et al. 2014), including waist-to-height ratio, waist-to-hip ratio, waist and hip girth. Different measures of adiposity have the benefit of increasing the reliability of the produced results.

2.1.1 Emotional overeating and childhood weight

About half of the studies to date have found no association between emotional overeating (EOE) and weight in children (Wardle, Marsland et al. 1992; Caccialanza, Nicholls et al. 2004; Jahnke and Warschburger 2008; Sleddens, Kremers et al. 2008; van Strien and Oosterveld 2008; Jollie-Trottier, Holm et al. 2009; Webber, Hill et al. 2009; Sleddens, Kremers et al. 2010; Svensson, Lundborg et al. 2011; Cao, Svensson et al. 2012; Snoek, Engels et al. 2013; McCarthy, Chaoimh et al. 2015). However the other half found significant positive associations between emotional overeating and BMI, indicating that children who tend to emotionally overeat more, also tend to have a higher BMI (Braet

and Van Strien 1997; Braet, Claus et al. 2008; Viana, Sinde et al. 2008; Joyce and Zimmer-Gembeck 2009; Webber, Hill et al. 2009; Parkinson, Drewett et al. 2010; Spence, Carson et al. 2011; Jansen, Roza et al. 2012; Hajna, LeBlanc et al. 2014; Domoff, Miller et al. 2015; dos Passos, Gigante et al. 2015; Steinsbekk and Wichstrom 2015). Importantly, no study has suggested a negative association between EOE and weight.

The majority of the studies used cross-sectional analyses. Of special interest are the three longitudinal studies examining the causal effect of emotional overeating on weight (Parkinson, Drewett et al. 2010; Snoek, Engels et al. 2013; Steinsbekk and Wichstrom 2015). As part of the Gateshead Millennium Study (UK), parents rated their children's (n=344) emotional overeating behaviour using the CEBQ at 5-6 years and again at 7-8 years. Additionally BMI was measured at both time points. Although there were no significant differences in EOE scores cross-sectionally between the low, middle and high BMI groups, EOE at 5-6 years significantly predicted increases in BMI from 5-6 years to 7-8 years. Analyses controlled for age, sex and birthweight and results suggested a causal link between emotional overeating and weight, such that children scoring high on emotional overeating at baseline were found to have a larger increase in BMI over a two-year follow up (Parkinson, Drewett et al. 2010). On the other hand, a similar sized study of Dutch adolescents (n=328) found no effect of emotional overeating on increases in BMI from 13 to 17 years (Snoek, Engels et al. 2013).

The third prospective study of EOE and BMI in a large sample of Norwegian children (n=760) found a significant positive association between EOE at four years and weight gain from four to eight years (Steinsbekk and Wichstrom 2015). This study controlled for other eating behaviours (such as satiety responsiveness and food responsiveness (SR and FR). After adjusting for the other eating behaviours, the relationship between EOE and weight was non-significant, although the prospective relationship between FR and weight remained. This finding suggests that the relationship between EOE and weight might be mediated by FR, such that only FR children are likely to emotionally overeat, and therefore to gain weight. To date these remain the only longitudinal studies examining the role of emotional eating on the development of weight in children.

Studies assessing the association between emotional overeating and weight were heterogeneous. The DEBQ, CEBQ and FAEHQ were used to measure eating behaviour. Even though these questionnaires are similar, variation in measurements complicate the comparisons between the different studies. Next to differences in questionnaire, age of participants varied greatly between the studies spanning an age range from one to 18 years. From the 22 studies, six included children under the age of five

(Jahnke and Warschburger 2008; Viana, Sinde et al. 2008; Joyce and Zimmer-Gembeck 2009; Cao, Svensson et al. 2012; Jansen, Roza et al. 2012; McCarthy, Chaoimh et al. 2015), which is an important developmental period when eating behaviours start to emerge. A list of studies investigating the association between EOE and weight is presented in Table 2.2.

Overall some evidence has been found suggesting an association between EOE and weight in childhood. However about half of the studies did not find significant associations. Further only one of the three longitudinal studies found a significant prospective relationship between EOE and weight gain. Most studies include children in early to middle childhood, and studies have tended to include a wide range of ages (without having explored interaction analyses with age) making it difficult to conclude if the relationship between EOE and weight differs by age. Lastly, the relationship between EOE and weight in toddlers is still relatively unexplored, with only one existing study of two year old (Irish) children (McCarthy et al, 2014).

Table 2.1 Studies investigating the association between emotional overeating eating and adiposity in childhood

Reference	Questionnaire	Adiposity measure	N	Age	Nationality	Design	Outcomes
Wardle et al (1992)	DEBQ*	BMI	846	11-18 years	UK	Cross-sectional	No association between EOE and BMI
Braet & Van Strien (1997)	DEBQ	Height and weight to calculate Ideal Body Weight groups (IDW)	292	9-12 years	Dutch	Cross-sectional	Increased EOE in obese children
Caccialanza et al (2004)	DEBQ	IOTF BMI cut-offs	312	12 years	Italian	Cross-sectional	No association between EOE and weight category
Braet et al (2008)	DEBQ	BMI categories	2474	7-18 years	Belgian	Cross-sectional	Overweight children and teenagers showed increased rates of EOE
Jahnke & Warschburger (2008)	DEBQ	BMI-SDS	142	3-6 years	German	Cross-sectional	No association between EOE and BMI
Sleddens et al (2008)	CEBQ*	BMI-SDS	132	6-7 years	Dutch	Cross-sectional	No association between EOE and BMI
van Strien & Oosterveld (2008)	DEBQ	BMI-SDS	769	7-12 years	Dutch	Cross-sectional	No association between EOE and BMI
Viana et al (2008)	CEBQ	BMI-SDS	240	3-13 years	Portuguese	Cross-sectional	EOE associated with higher BMI
Cunha et al (2009)	CEBQ	BMI-SDS	321	9-12 years	Portuguese	Cross-sectional	EOE associated with higher BMI
Jollie et al (2009)	DEBQ	CDC weight categories	232	8-12 years	Native American	Cross-sectional	No association between EOE and higher BMI
Joyche & Zimmer-Gembeck (2009)	CEBQ	CDC weight categories	211	4-8 years	Australian	Cross-sectional	EOE and FR combined associated with higher BMI
Webber et al (2009)	CEBQ	BMI-SDS	263	7-12 years	UK	Cross-sectional	EOE associated with higher BMI
Parkinson et al (2010)	CEBQ	BMI	344	5-6 years 6-8 years	UK	Longitudinal	Longitudinal association between EOE and BMI

Spence et al (2011)	CEBQ	CDC weight categories	1730	4-5 years	US	Cross-sectional	EOE associated with higher BMI
Svennson et al (2011)	CEBQ	BMI-SDS	174	1-6 years	Swedish	Cross-sectional	No association between EOE
Cao et al (2012)	CEBQ	BMI-SDS	219	18 months	Chinese	Cross-sectional	No association between EOE and BMI
Snoek et al (2013)	DEBQ	BMI-SDS	328	13-15 years	Dutch	Longitudinal	No association between EOE and BMI
Braden et al (2014)	CEBQ	CDC weight categories	106	8-12 Years	US	Cross-sectional	No association between EOE and BMI
Hajna et al (2014)	Family Eating and Activity Questionnaire	BMI, Waist-to-height ratio, waist-t0-hip ratio, waist and hip girth	431	12 years	Canadian	Cross-sectional	EOE associated with higher BMI, Waist-to-height ratio, waist-t0-hip ratio, waist and hip girth
McCarthy et al (2014)	CEBQ	IOTF BMI cut-offs	1189	2 years	Irish	Cross-sectional	No association between EOE and BMI
Dos Passos et al (2015)	CEBQ	WHO BMI cut-offs	335	7 years	Brazilian	Cross-sectional	EOE associated with higher BMI
Domhoff et al (2015)	CEBQ	BMI-SDS	1002	4 years	US	Cross-sectional	EOE associated with higher BMI
Steinsbekk & Wichstrom (2015)	CEBQ	BMI-SDS	760	4 years 8 years	Norwegian	Longitudinal	Association between EOE and BMI, association did not survive controlling for other eating behaviours

*Abbreviations: DEBQ; Dutch Eating Behaviour Questionnaire, CEBQ; Child Eating Behaviour Questionnaire

2.1.2 Emotional under-eating and childhood weight

Fewer studies have examined the relationship between emotional undereating (EUE) and weight. All of these studies have measured EUE using the CEBQ, the only available measure of emotional under-eating in children. Four of 11 cross-sectional studies found a significant negative association between EUE and weight (Viana, Sinde et al. 2008), Cunha et al, 2010, (Jansen, Roza et al. 2012; Domoff, Miller et al. 2015) suggesting that children who tend to emotionally under-eat, tend also to be thinner. The

remaining seven cross-sectional studies found no significant relationship (Sleddens, Kremers et al. 2008; Webber, Hill et al. 2009; Parkinson, Drewett et al. 2010; Spence, Carson et al. 2011; Svensson, Lundborg et al. 2011; Cao, Svensson et al. 2012; dos Passos, Gigante et al. 2015; McCarthy, Chaoimh et al. 2015). There has only been one longitudinal study assessing the impact of EUE on weight gain, which did not find a significant association (Parkinson, Drewett et al. 2010).

In comparison to the research examining the relationship between EOE and weight, studies of EUE have all used the CEBQ increasing the homogeneity of the conducted studies. In keeping with research examining EOE and weight, the age range included in most of these studies was wide, spanning from 18 months to 13 years. This makes it difficult to understand if the association is moderated by age. Another possibility is that null findings may have missed an association in sub-sample of the children who are younger or older. Lastly, and importantly, no study has found a positive association between EUE and weight. Additional studies are necessary that focus on a more narrow age range of children, and take a prospective approach.

Table 2.2 Studies investigating the association between emotional under-eating eating and adiposity in childhood

Reference	Questionnaire	Adiposity Measure	N	Age	Nationality	Design	Outcomes
Sleddens et al (2008)	CEBQ	BMI-SDS	132	6-7 years	Dutch	Cross-sectional	No association between EUE and BMI
Viana et al (2008)	CEBQ*	BMI-SDS	240	3-13 years	Portuguese	Cross-sectional	EUE associated with lower BMI
Cunha et al (2010)	CEBQ	BMI-SDS	321	9-12 years	Portuguese	Cross-sectional	EUE associated with lower BMI
Webber et al (2009)	CEBQ	BMI-SDS	263	7-12 years	UK	Cross-sectional	EUE not associated with BMI
Parkison et al (2010)	CEBQ	BMI-SDS	344	5-6 years 6-8 years	UK	Longitudinal	No association between EUE and BMI
Spence et al (2011)	CEBQ	CDC weight categories	1730	4-5 years	US	Cross-sectional	No associations between EUE and weight category
Svennson et al (2011)	CEBQ	BMI-SDS	174	1-6 years	Swedish	Cross-sectional	No association between EUE and BMI
Cao et al (2012)	CEBQ	BMI-SDS	219	18 months	Chinese	Cross-sectional	No association between EUE and BMI
Jansen et al (2012)	CEBQ	BMI-SDS	4984	4 years	Dutch	Cross-sectional	EUE associated with lower BMI
McCarthy et al (2015)	CEBQ	IOTF BMI cut-offs	1189	2 years	Irish	Cross-sectional	No association between EUE and weight category
Domhoff et al (2015)	CEBQ	BMI-SDS	1002	4	US	Cross-sectional	EUE associated with lower BMI
Dos Passos et al (2015)	CEBQ	WHO BMI cut-offs	335	7 years	Brazilian	Cross-sectional	No association between EUE and weight category

*Abbreviations: CEBQ; Child Eating Behaviour Questionnaire

2.1.3 The relationship between EOE and EUE

The relationship between EOE and EUE has received limited attention. Somewhat surprisingly, studies have found significant *positive* correlations (ranging from 0.21 to 0.41) between the two constructs in children aged three to eight years (Wardle, Guthrie et al. 2001; Sleddens, Kremers et al. 2010; Domoff, Miller et al. 2015); indicating that children who tend to emotionally overeat, tend also to under-eat in response to emotions, despite the two behaviours showing opposite relationships with adiposity. The extent to which these behaviours are different expressions of the same underlying trait (i.e. the tendency to have one's appetite up- or down-regulated by stress), or represent different phenomena, is unknown.

2.2 Aetiology of emotional eating

Due to its potentially crucial role in the development of obesity, researchers have been interested in understanding the aetiology of emotional eating behaviour. Section 2.2 summarises the main theories that have been put forward to explain how emotional eating develops, and research from twin studies regarding the aetiology of emotional eating in adulthood are discussed. Lastly, specific environmental factors associated with emotional overeating in children are considered.

2.2.1 Theories of emotional eating

Two main theories have been formulated to explain the development of emotional overeating. The Psychosomatic Theory (Kaplan and Kaplan 1957) proposes that obese individuals have not learned to distinguish successfully between the arousal caused by hunger, and negative emotion; possibly because of classical conditioning in early life, putting an emphasis on the influence of early eating and feeding influences such as parenting feeding strategies. For example, parents who use food as a reward or to induce a positive mood and distract from negative emotions are thought to teach children to engage in emotional eating, through conditioning. The hypothesis is that if consumption of food often follows the onset of negative feelings, a classically conditioned hunger response to stress can develop (Bruch 1964).

The Internal/External theory (Schachter, Goldman et al. 1968) suggests a slightly different basis for EOE. It proposes that healthy weight individuals tend to decrease their food intake in stressful situations, in response to internal physiological stress cues. On the other hand, obese individuals' appetites are hypothesised to be abnormal in that they are not affected by stress. The theory still predicts that obese individuals eat more than normal weight individuals during times of stress, but due to the inability to respond 'normally' to stress cues insofar as they do not downregulate their

intake (van Strien and Ouwens 2003). Such aberrations in biology could be innate or learned. There has been some support for both theories (Psychosomatic Theory: Bruch 1975, Wooley 1984, Heatherton, Striepe et al. 1998; Internal/External theory: Schachter, Goldman et al. 1968, Herman and Polivy 1984, Willner, Muscat et al. 1992). Recent evidence framed the development of emotional overeating as acquired through Pavlovian learning, in line with the Psychosomatic Theory, and supporting the notion that the behaviour is indeed developed and maintained through environmental influences (Jansen, Havermans et al. 2011; Bongers, van den Akker et al. 2015).

Restraint Theory came along somewhat later, and offered a slightly different explanation for the association between emotional eating and obesity. Restraint Theory is based on the idea that individuals have a unique satiation point that their bodies are naturally trying to achieve. Dieting (restrained eating) prohibits this satiation point from being attained, which can be successfully achieved during 'normal' situations with few other competing cognitive or physiological burdens. However, stress and negative emotions interfere with the ability to cognitively restrain and inhibit eating, leading restrained eaters to become disinhibited and overconsume (Nisbett 1972; Herman and Mack 1975). However, Restraint Theory seems difficult to adapt for young children, who are unlikely to be exerting any restraint over their food intake.

2.3 Twin studies of emotional eating

Twin studies provide a powerful method for understanding the extent to which individual differences in a characteristic such as emotional eating are shaped by genes versus environmental influences. Importantly, as highlighted in Chapter 1, twin analyses can also provide insight into the relative importance of two different types of environmental influence – aspects of the environment that are completely shared by two twins in a pair (shared environmental effects), and environmental influences that are unique to each individual twin (unique environmental effects). There have been a number of twin studies of the aetiology of emotional overeating in adults; these are presented in Table 2.3.

Emotional overeating in adult twins has been measured using the adult version of the Dutch Eating behaviour Questionnaire (DEBQ) (Vanstrien, Frijters et al. 1986) and the revised version of the Three Factor Eating Questionnaire (R21) (TFEQ) (de Lauzon, Romon et al. 2004). Both questionnaires use self-report measures of emotional overeating and have been validated (Cappelleri, Bushmakin et al. 2009; Cebolla, Barrada et al. 2014). The items in the emotional eating scales of each questionnaire are shown in Appendix 2.

A study of Swedish male twins (MZ: 456 pairs; DZ: 326 pairs) aged 23-29 years, suggested that 60% of the variation in emotional overeating, measured with the TFEQ, was explained by genetic effects. Non-shared environmental effects explained the remaining 40%, with no detectable effect of the shared environment (0%) (Tholin, Rasmussen et al. 2005). A following study including adult twins from the UK and Finland confirmed that the shared environment did not contribute to variation in emotional eating, measured with the TFEQ (Keskitalo, Tuorila et al. 2008). Participants were between 17 and 82 years of age (MZ: 314 pairs, DZ: 327 pairs), and estimates varied between men and women. In women, a larger proportion of individual differences in emotional eating were explained by genetic effects (45% UK, 31% Finland); while they were non-significant for the men. For both sexes the majority of variation was explained by non-shared environmental effects. However, there were far fewer men (n=231) than women (n=1095), and estimates derived from smaller sample sizes have larger confidence intervals, making them less reliable. Due to the small sample of male participants, the confidence intervals for the genetic effects were wide, but heritability could have been as high as 45% (UK males = 0% - 47%; Finish males = 0% - 38%) (Keskitalo, Tuorila et al. 2008). More recently a study of adult twins (mean age: 38.1 years) from Korea (MZ: 441 pairs, DZ: 124 pairs) reported moderate genetic effects (32%), but again the majority of variation in emotional eating was explained by non-shared environmental factors, in keeping with the other adult studies (Sung, Lee et al. 2010).

A recent study investigated genetic and shared environmental contributions to variation in emotional overeating using a slightly different twin design that takes advantage of identical twins that have been raised apart (Briley and Tucker-Drob 2013). Comparing MZ twins who are reared together, with MZ twins reared apart provides direct information about the importance of the shared environment. MZ twins reared-apart share only their genes; MZ twins reared together share both their genes and aspects of their environment. This comparison therefore makes it possible to directly estimate the contributions of genes and shared environments – greater similarity between the MZs reared together versus those reared apart reflects the additional shared environmental effects for those reared together, that do not contribute to similarity for those reared apart. Emotional overeating was measured with the TFEQ in 22 MZ twins raised apart (MZA) and 37 MZ twins raised together (MZT). MZAs and MZT differed significantly by age, with the MZA group being significantly older (Mean age: 50.7; MZT mean age: 28.7). Both types of twins were correlated for EOE, but there was no difference between the two types of twins in their similarity. These results showed that genetics played a moderate role in explaining individual differences in EOE (55%), and that the shared environment did not contribute at all to variation in this trait (Elder, Neale et al. 2012).

In conclusion, twin studies of the genetic and environmental influences on EOE have suggested that individual differences in EOE are explained to some extent by genetic variation, but the majority of differences between people are explained by aspects of the environment that are unique to each person. Large twin studies have the power to calculate precise estimates, with narrow confidence intervals. However most of the studies were limited in sample size, producing less reliable estimates, especially when examining sex differences. Furthermore, the large age range of the studies adds to the heterogeneity of the findings. Genetic and environmental influences on individual differences in behavioural traits have been found to differ across the lifespan (Bergen, Gardner et al. 2007). In keeping with these, previous longitudinal twins studies of BMI have also shown that heritability estimates are not stable, but change considerably over time (Haworth, Carnell et al. 2008; Briley and Tucker-Drob 2013). The wide age range of these studies therefore complicates interpretation of the findings. Individual differences in other appetitive traits, such as satiety and food responsiveness in childhood have already been investigated using the twin method, providing evidence for strong genetic effects (Carnell, Haworth et al. 2008; Llewellyn, van Jaarsveld et al. 2010). Similarly twin studies of EOE in children would help to elucidate its aetiology, especially given that it emerges and is measurable during early childhood. Furthermore, no twin study to date (in adults or children) has investigated the genetic and environmental contributions to EUE. Additionally, longitudinal twin studies of emotional eating starting early in childhood are needed to further understanding of the aetiology of emotional eating and its development across childhood.

Table 2.3 Twin Studies investigating genetic and environmental contribution to EOE in adults

Study	Questionnaire	Sample	Age	Nationality	Estimates
Tholin et al (2005)	TFEQ-R21*	MZ*: 456 DZ*: 326	23-29 years	Swedish	A*: 60% C*: 0% E*: 40%
Keskitalo et al (2008)	TFEQ-R21	MZ: 314 DZ: 327	17-82 years	UK & Finnish	A: 9-45% C: 0% E: 55-91%
Sung et al (2010)	DEBQ*	MZ: 441 DZ:124	20-65 years	Korean	A: 25% C: 0% E: 75%
Elder et al (2012)	TFEQ-R21	MZA: 22 MZT: 37	18-72 years	US	A: 55% E: 55%

*Abbreviations: FFEQ-R21: Three Factor Eating Questionnaire, MZ: Monozygotic, DZ: Di-zygotic, A: Latent factor, genetic effects, C: latent factor, shared-environmental effects, E: latent factor, unique environmental effects, DEBQ: Dutch Eating Behaviour Questionnaire

In addition to twin research, recent molecular genetic work with adults has suggested that emotional overeating mediates some of the association between genetic risk for obesity (indexed using a composite score of the 97 obesity-associated SNPs) and BMI, indicating that emotional overeating may be a behavioural phenotype that sits on the pathways between 'obesity genes' and adiposity (Konttinen, Llewellyn et al. 2015).

2.4 Environmental and individual influences on emotional eating

The relative contributions of genetic and environmental influences on individual differences in emotional eating in childhood are unknown. However, previous research has aimed to identify specific factors at different levels of influence that shape emotional eating in childhood, from individual child-level factors, to parent-level factors, and the wider family environment. These are discussed below.

2.4.1 Child factors

2.4.1.1 Emotion regulation

The ability to regulate one's emotions has been suggested to be related to individual differences in emotional eating in both adults and children. For example, children engaging in binge eating behaviour were found to engage in maladaptive emotion regulation strategies (Czaja, Rief et al. 2009). In addition, emotion regulation training resulted in a decrease in binge eating disorder in adult men (Clyne and Blampied 2004). Other research has shown that participants instructed to repress negative

emotions ate more in comparison to participants allowed to express emotions freely, suggesting that maladaptive emotion regulation impacts on food intake (Evers, Stok et al. 2010). Recently, a study investigating the relationship between emotion regulation, emotional eating and intake of energy rich foods in a large sample of Chinese teenagers suggested that suppression of emotions is associated with greater emotional eating; and emotional eating mediated the relationship between emotion regulation and intake of energy dense food (Lu, Tao et al. 2016).

2.4.1.2 Food responsiveness

Food responsiveness (FR) tends also to be positively associated with emotional overeating in children (r=0.49-0.54) such that those who are more food responsive tend also to emotionally overeat (Wardle, Guthrie et al. 2001; Sleddens, Kremers et al. 2008). Children who respond more positively to food cues might be more likely to be rewarded with food by their parents, and hence might be prone to emotionally overeat. It makes sense that parents are more likely to try and soothe a child with food, if that child responds positively to food. On the other hand, a child who shows little interest in food is likely to be comforted via other strategies. This hypothesis has received some support insofar as FR has been associated with the same parental feeding strategy (restriction) as EOE in a sample of over 4000 Dutch children, suggesting that both might be influenced by or elicit similar parenting (Jansen, Roza et al. 2012). Additionally a genetically sensitive study of a sample of five year olds (N=1718) suggested that children carrying the minor A allele of the obesity risk gene FTO are less food responsive and also show greater emotional control. These findings provide some support for the behavioural susceptibility model of obesity, and suggest a role for EOE, alongside FR, as a behavioural mediator of genetic susceptibility to weight (Velders, De Wit et al. 2012).

2.4.2 Parent level factors

2.4.2.1 Parental feeding styles

The Psychosomatic Theory of emotional eating hypothesises that children learn to engage in emotional eating from certain parenting styles (Kaplan and Kaplan 1957, Bruch 1964, Schachter, Goldman et al. 1968). In particular, children might learn to regulate their negative emotions from their parents, via feeding practices. Previous studies have suggested that certain parental feeding practices are associated with higher EOE in children. Children whose parents actively controlled their emotions through feeding (so-called 'instrumental feeding') were found to engage more in EOE (Blissett, Haycraft et al. 2010; Braden, Rhee et al. 2014; Tan and Holub 2015). Furthermore, a longitudinal study suggested that restrictive feeding and using food as a reward in order to control three to five year old

children's food intake, was associated with child emotional overeating two years later (Farrow, Haycraft et al. 2015). These findings propose that parents who use food as a reward and exert high levels of control over their child's eating might encourage the development of emotional overeating behaviour in their children.

2.4.2.2 General parenting styles

Next to specific feeding strategies, other more general parenting styles were found to be associated with emotional eating in childhood. Adolescents experiencing low maternal support but high psychological control were found to engage more in emotional overeating (Snoek, Engels et al. 2007). In addition, maternal rejection and negative affect have both been hypothesized to disrupt a child's ability to regulate his or her emotions, potentially predisposing the child to emotionally overeat (Rodgers, Paxton et al. 2014; Vandewalle, Moens et al. 2014). Maternal attachment anxiety has also been associated with increased emotional feeding practices and child emotional eating behaviour (Hardman, Christiansen et al. 2016). In line with the Psychosomatic Theory and the Internal/External Theory existing evidence supports the idea that parenting influences the development of emotional eating behaviour. However, previous studies have focussed on school aged children. Yet variation in eating (or feeding) behaviour is measurable from early postnatal life, and parents develop their feeding strategies very early on. Future longitudinal studies that investigate the impact of parental feeding behaviour from very early in childhood and beyond would help to shed light on the relationship between parental feeding styles and children's tendency to emotionally eat (DiSantis, Hodges et al. 2011).

2.4.3 Family level factors

A stressful and chaotic home environment has been associated with childhood obesity. One of the mediators of this relationship might be EOE. A child is more likely to learn to engage in EOE in an environment that requires strategies to manage stress and negative emotion (Gundersen, Mahatmya et al. 2011). A recent meta-analysis provided evidence that maternal stress predicted increased child BMI. Associations were especially strong if the stressor also affected the child (Tate, Wood et al. 2015). Further research is needed to fully understand the interrelationship between stress in the home, parental feeding strategies and child emotional eating.

2.5 Conclusions

Traditional models of emotional eating and weight have not produced one unifying theory. Previous models suggest that biological and environmental factors interact to confer vulnerability towards

emotional over- and under-eating. In times of stress and negative emotions, individual differences in learned coping strategies and biological signalling appear to push some individuals to overeat, contributing to weight gain.

Developmental studies of emotional eating and weight development in young children would provide new insight into the relationship between this behaviour and weight gain. In addition, research is needed to understand the aetiology of emotional over and undereating in childhood. In general, EUE has received considerably less attention in the literature than EOE, in terms of its impact on weight and its aetiology (with no twin studies to date). Additionally the nature of the relationship between EOE and EUE is largely unknown.

So far twin research investigating the relative contributions of genes and the environment to individual differences in emotional eating is sparse. Previous studies have focused entirely on adults and have found evidence for both genetic and non-shared environmental contributions to variation in emotional overeating, although none of the studies detected any effect of the shared environment. There are no existing twin studies investigating the contribution of genes and environments to individual differences in emotional over or undereating in childhood. In addition, there have not been any longitudinal twin studies to understand how the relative influence of genes and environments changes as children get older.

3 Aims of the thesis

Emotional eating behaviour develops early in life and has been proposed to play a causal role in the development of obesity. Previous studies have explored the relationship between emotional eating and weight; but most studies have focused on school-aged children, and included a wide range of ages. There are very few studies of the preschool period when these tendencies to emotionally over- and under-eat start to emerge. In addition, emotional over- and under-eating tend to correlate positively in studies, but the nature of the relationship between them remains largely unexplored.

Another important but unexplored relationship is that between food responsiveness (FR) and emotional overeating. These two traits tend to correlate positively to a moderate extent, insofar as more food responsive children tend also to emotionally overeat. It is possible that emotional overeating is a behavioural phenotype that mediates some of the association between food responsiveness and weight, but this has never been explored.

The aetiology of emotional eating has also been of interest to researchers, because of its relationship with weight. Twin studies are ideal for broadly quantifying the relative importance of genetic and environmental influences underlying individual differences in emotional eating. But existing studies have focused entirely on adults, with no paediatric studies to date. This is an important gap in the literature, because the relative influence of the shared family environment may be much greater when children are younger, and parents usually play an important role in the feeding interaction. This thesis aims to fill in some of these gaps in the existing research base.

3.1 Overall aim

In this PhD I will examine the aetiology of emotional over and under-eating in early childhood, and establish their relationships with adiposity.

3.2 Specific aims

1) To estimate genetic and environmental contributions to individual differences in emotional over and under-eating in early childhood.
    a. To test for bias in parent-reported measures of child emotional eating in relation to perceived zygosity, using a misclassified zygosity design. This will ensure that estimates of genetic and environmental influences on these measures derived from the twin method are reliable.

b. To assess the genetic and environmental contributions to emotional *overeating* during toddlerhood (16 months) and early childhood (five years) using a longitudinal twin study.
c. To assess for the very first time the genetic and environmental contributions to emotional *undereating* in early childhood (five years) using a twin design
d. To investigate the association between emotional over and under-eating in early childhood (age five), and the extent to which they share a common or distinct aetiology using the twin design.

2) To examine together the interrelationships between child emotional over- and under-eating in early childhood (five years), and child factors (such as emotion regulation and food responsiveness), parental factors (such as instrumental and emotional feeding and their own tendency to emotionally overeat), and wider family factors (such as stress in the home).

3) To explore the interaction between genetic and environmental factors in the expression of emotional over- and under-eating in childhood.
    a. To test for gene-environment interactions in the expression of emotional over- and under-eating behaviour by testing if genetic and environmental influences on emotional over- and under-eating vary according to different environmental exposures (using a score of environmental risk, that aggregates factors identified in aim 2).

4) To establish the relationships between emotional over- and under-eating and adiposity during toddlerhood (16 months) and early childhood (five years), and the interrelationships with food responsiveness
    a. To examine whether there is a cross-sectional association between emotional overeating at 16 months and BMI at two years.
    b. To examine whether there are cross-sectional associations between emotional overeating and emotional under-eating and BMI at five years.
    c. To examine the longitudinal relationship between emotional overeating at 16 months with change in BMI from two to five years.

d. To test if emotional overeating mediates the cross-sectional and longitudinal associations between food responsiveness and BMI at 16 months and five years

To date aim 1 has been partially achieved. Results are presented in Chapters 6 and 7 of this report. Planned work to address the remaining aims is outlined in Chapter 8. A timetable for the completion of my PhD is presented in Chapter 9.

4 Methods

4.2 The Gemini birth cohort

4.2.1 Overview

The Gemini cohort study was set up by the Health Behaviour Research Centre, Department of Epidemiology and Public Health, University College London in 2008. Its main aims are to: (1) investigate the genetic and environmental influences on weight gain and appetite in childhood, (2) identify modifiable risk factors for excessive early weight gain, and (3) establish a database of early developmental exposures to assess the contributors to long-term health (van Jaarsveld, Johnson et al. 2010).

4.2.2 Recruitment, description and representativeness of the Gemini sample

In January 2008 all families (N=6754) with twins born between March and December 2007 in England and Wales were invited to enrol in the study by the Office of National Statistics. Half of the families (N=3425, 51%) agreed to be contacted by the research team. Between February and April 2008 consent forms and baseline questionnaires were sent out to these families and 2402 (36%) completed and returned the baseline questionnaire. At baseline, one third of twin pairs (N=2402) were male (N=785, 32.7%), one third were female (N=801, 33.3%) and one third were opposite sex (N=816, 34.0%). Since its initiation the Gemini study has collected data on child weight, eating behaviours, parental feeding strategies and other home environmental factors at various time-points, largely by questionnaire. A schematic overview of the Gemini data collection phases is shown in Table 4.1. This thesis will focus on questionnaire-based measures of eating behaviour (the Baby Eating Behaviour Questionnaire [BEBQ]; the Child Eating Behaviour Questionnaire [CEBQ], and its version for toddlers, the Child Eating Behaviour Questionnaire Toddler version [CEBQ-T]), parental feeding styles (Parental Feeding Style Questionnaire [PFSQ]), stress in the home environment (Confusion, Hubbub and Order Scale [CHAOS]) and child related characteristics (e.g. emotion regulation measured using the Strength & Difficulties Questionnaire [SDQ] and the Child Behaviour Questionnaire [CBQ]).

Table 4.1 Schematic overview of the assessment points and measures in Gemini that are used in this thesis

	Twin Age	**0-1 years**	**1-2 years**	**2-3 years**	**2-3 years**	**3-4 years**	**5-6 years**
Assessment	Date	2007-2008	2008-2009	2009-2010	2009-2010	2010-2012	2012-2013
	Response rate n [% of baseline]	2402 (100%)	1930 (80%)	1364 (57%)	1126 (47%)	1119 (47%)	1087 (45%)
Socio-demographics	Parental education	x					
	Parental ethnicity	x					
Twin characteristics	Date of birth	x					
	Birth weight	x					
	Anthropometrics (height and weight[1])	x	x	x		x	x
	DNA				x		
	Number of siblings in household	x	x			x	x
Home environment	Home food environment[2]					x	
	Stress in the home (CHAOS*)						X
	Parental feeding styles	x	x	x			X
Eating Behaviour	BEBQ*	x					x
	CEBQ-T*		x				
	CEBQ*						x
Child Psychometrics	CBQ*						x
	SDQ*						x

*Abbreviations: CHAOS, Confusion, Hubbub and Order Scale; BEBQ, Baby Eating Behaviour Questionnaire; CEBQ-T, Child Eating Behaviour Questionnaire-Toddler; CEBQ, Child Eating Behaviour Questionnaire; CBQ, Child Behaviour Questionnaire; SDQ, Strength and Difficulties Questionnaire

[1] Height and weight data have been collected every three months since 2009, when the twins were approximately two years old

[2] Detailed information about the Home environment was collected via telephone interview when the twins were three-four years old.

Compared with national twin statistics, Gemini twins were representative regarding sex, zygosity, gestational age and birthweight (see Table 4.2). However, compared to population statistics Gemini mothers were slightly older and healthier insofar as they smoked less (12.7% versus 21%) and had a slightly lower BMI than the population mean. Rates of vegetable and fruit consumption were

comparable between Gemini parents and national statistics. White-British families were over-represented. The baseline parental characteristics in comparison to national health statistics are shown in Table 4.3.

Table 4.2 Characteristics of Gemini twins compared to National twin statistics[1].
Table adapted from van Jaarsveld et al. (2010).

	Gemini Cohort (Baseline)	**National twin statistics[1]**
	N (%)	%
Sex of twin pair		
Male	785 (32.7%)	32.1%
Female	801 (33.3%)	32.8%
Opposite sex	816 (34.0%)	35.1%
Pre-term (<37 weeks)	1045 (43.5%)	40%
	Mean (SD)	Mean
Gestational age, mean (SD)	36.20 (2.48)	37
Birth weight, mean (SD)	2.46 (0.54)	2.50

[1] Office for National Statistics (2006). Birth Statistics Series FM1 no.35. Review of the Registrar General on births and patterns of family building in England and Wales. Newport. (Numbers are for twin births in 2006).

Table 4.3 Characteristics of Gemini families compared to National health statistics[1].
Table adapted from van Jaarsveld et al. (2010).

Families	Gemini Cohort (Baseline)	National health statistics
	Mean (SD)	Mean
Age at twins' birth (years)		
Mother	33.6 (5.2)	29.5[1]
Father	36.4 (6.2)	
BMI in kg/m^2		
Mother	25.1 (4.8)	26.8[2]
Father	26.4 (3.9)	27.1[2]
	N (%)	%
Mother's Ethnicity		
White-British	2089 (87.8%)	72.6%[1]
Non White British	311 (12.9%)	21.9%
Not known	2 (0.1%)	
Current Smoker		
Mother	306 (12.7%)	21.0%[1]
Father	466 (19.4%)	24.0%[1]
At least 5 portions of fruit or vegetables a day		
Mother	790 (32.9%)	31.0%[1]
Father	663 (27.6%)	27.0%[1]

[1] Health Survey for England 2007 Volume 1. Health lifestyles: knowledge, attitudes and behaviour. Ed R. Craig & N. Shelton. The health and social care Information Centre, 2008.
[2] BMI calculated from self-reported height and weight.

Gemini is a longitudinal study and data are still being collected. Table 4.4 shows the characteristics of the Gemini sample at baseline, and for families who provided follow up data at 16 months and five years. This thesis largely analyses the data collected at 16 months and five years.

Table 4.4 Characteristics of Gemini twins at baseline, 16 months and five years.

	Gemini Cohort (Baseline)	Gemini 16 months	Gemini 5 years
	N (%)	N (%)	N (%)
N of children	4804	3784	2174
Sex			
Male	2386 (49.7%)	1860 (49.2%)	1053 (48.4%)
Female	2418 (50.3%)	1924 (50.8%)	1121 (51.6%)
	Mean (SD)	Mean (SD)	Mean (SD)
Gestational age, mean	36.20 (2.48)	36.21(2.47)	36.25 (2.44)
Birth weight, mean	2.46 (0.54)	2.47 (0.54)	2.46 (0.54)
Maternal age at birth	33.6 (5.2)	33.4 (5.0)	33.8 (4.7)
Age at data collection	8.17 (2.18)	15.82 (1.15)	5.15 (0.13)

4.3 Zygosity assignment

One prerequisite of the twin method is the successful identification of MZ and DZ twin pairs. This can be an issue in large cohorts and especially when infants or very young children are involved. Due to the price of DNA genotyping, questionnaires to assess zygosity status are mostly used. Several questionnaires have been shown to be valid and reliable for use in children (Goldsmith 1991; Price, Freeman et al. 2000; Rietveld, van Der Valk et al. 2000). The best way to test the validity of a zygosity questionnaire is to compare the measure against the results of DNA markers. Having reliable measures of zygosity is crucial for conducting successful twin research, especially in the light of the evidence suggesting that up to a third of parents misclassify their identical twins as non-identical due to misinformation from health professionals (Ooki, Yokoyama et al. 2004; van Jaarsveld, Llewellyn et al. 2012).

4.4 Zygosity testing in Gemini

All opposite sex twins (816 pairs) were classified as DZ. At baseline, families with same-sex twins (1586 pairs) were asked to complete a questionnaire to determine the zygosity of their twins (Price, Freeman et al. 2000) when they were on average eight months old (SD: 2.1). The 20 items of the questionnaire examine general physical resemblance, such as eye and hair colour, teeth growth and the ability of others (friends and family members) to distinguish the siblings. 934 families (58.9%) completed the same questionnaire again when the twins were on average 28.8 months old (SD=3.3). Mean questionnaire scores were calculated, creating values between 0 and 1 for each twin pair, and scores

were used to determine zygosity. In line with Price et al (Price, Freeman et al. 2000), lower scores indicate greater similarity, whereas higher scores indicate difference. Twin pairs scoring 0.64 and lower were classified as MZ. Twin pairs scoring 0.70 and above were classified as DZ. Scores falling between 0.64 and 0.70 were noted as 'unclear'. From 934 families who answered the questionnaires at both time points, 66 pairs were of uncertain zygosity. Of the remaining 868 pairs, 95.3% (827 pairs) of the zygosity assignment matched between the two time points.

4.4.1 Zygosity testing using DNA

Furthermore, 1127 families provided DNA samples for both twins in order for them to be genotyped for obesity-related single nucleotide polymorphisms. Of these, 81 twin pairs were randomly selected for zygosity testing using their DNA. These pairs were used to validate the zygosity questionnaire. In addition, some families elected to have their DNA used for zygosity testing (n=117) and we tested a further 112 pairs of the 1127 families who could not be classified using questionnaire data but who had provided DNA samples (for 88 pairs there was a mismatch between the two questionnaires, for 24 pairs they were missing the second zygosity questionnaire). The process of zygosity testing with DNA involves detecting multiple tandem-repeat copies of 10-15 base pairs sequences, using hyper-variable minisatellite DNA probes. These tandem repeat copies can be found all over the genome and are identical for MZ twins, but differ for DZ twins. (Hill and Jeffreys 1985; Jeffreys, Wilson et al. 1985).

For the 81 randomly selected pairs, genotyping and questionnaire classification matched in all cases. Results from the questionnaire (all pairs for whom questionnaire data only was used to allocate zygosity, n=1239) and the DNA testing (all pairs who were zygosity tested using DNA which included the random sample and the parent-requested sample and the additional pairs we tested in order to classify them, n=310) were combined to provide the most accurate zygosity assignment for the Gemini sample. A total of 749 twin pairs (31.2 %) were classified as MZ and 1616 (67.3%) twin pairs were classified as DZ (including 816 opposite sex DZ twins), based on the questionnaire and DNA results. For a further 37 pairs (1.5%) zygosity could not be established, as questionnaire results were unclear and no DNA was provided. Table 4.5 shows the number of MZ and DZ pairs at baseline, 16 months and 5 years. The total number of pairs is declining as the study continues, but importantly the ratio of MZ and DZ twin pairs stays similar.

Table 4.5 Zygosity at baseline, 16 months and 5 years.

	Zygosity established from questionnaire and DNA	16 months	5 years
	Frequency (%)	Frequency (%)	Frequency (%)
MZM*	352 (14.7)	290 (15.0)	181 (16.7)
DZM*	409 (17.0)	325 (16.8)	172 (15.8)
MZF*	397 (16.6)	326 (16.9)	181 (16.7)
DZF*	391 (16.3)	316 (16.4)	209 (19.2)
DZO*	816 (34.0)	644 (33.4)	335 (30.8)
Unknown	37 (1.5)	30 (1.6)	9 (0.8)
Total	2402	1931	1087

*Abbreviations: MZM: male-male monozygotic pairs; DZM: male-male dizygotic pair; MZF: female-female monozygotic pair; DZF: female-female dizygotic pair; DZO: opposite sex dizygotic pair

4.5 Measuring adiposity

Weight and length at birth were taken from the children's personal health records ('the red book'). When the twins were approximately two years of age, electronic weighing scales and height charts were sent to all participating families to aid measurements and ensure standardisation of the data collection. From then on parents were asked to log their children's heights and weights every three months on the Gemini online platform (van Jaarsveld, Johnson et al. 2010). BMI measurements were calculated using children's heights and weights (weight (kg) / height $(m)^2$). BMI and weight scores were standardised to create BMI standard deviation scores (BMI-SDS) to take account of the age and sex of the child, using 1990 UK reference data, in the LMSgrowth macro in Microsoft Excel (Cole 1990; Freeman, Cole et al. 1995).

The use of BMI as an adiposity indicator in children has been widely discussed. BMI gives only an indication of adiposity, as it does not measure actual body fat (Prentice and Jebb 2001). For example individuals with high muscle mass (and therefore high weight) have a high BMI, but low body fat (Ode, Pivarnik et al. 2007). In childhood, the use of BMI has been criticised especially as periods of growth and development were found to result in unreliable BMI measures with moderate association of actual percentage of body fat (Demerath, Schubert et al. 2006). However, BMI-SDS are far more reliable. Height and weight data are easy to collect, and are a cost effective option in large datasets

like Gemini (Reilly, Dorosty et al. 2000; Freedman and Sherry 2009). When investigating weight below the age of two standardized weight scores were used instead of BMI because height cannot be reliably measured before age two years.

4.6 Measuring emotional over- and under-eating in children

Emotional over- and under-eating were measured using the Child Eating Behaviour Questionnaire (CEBQ), a parent report questionnaire designed to assess a range of weight-related eating behaviours in children (Wardle, Guthrie et al. 2001). The questionnaire consists of 35 items and parents use a 5-point Likert scale to rate their child's behaviour. The 35 items cluster into eight distinct eating behaviours: Satiety Responsiveness (SR), Food Responsiveness (FR), Emotional Overeating (EOE), Emotional Undereating (EUE), Food Fussiness (FF), Desire to drink (DD), Enjoyment of Food (EF) and Slowness in Eating (SE). The EOE and EUE subscales have high Cronbach's alphas (0.72-0.79 and 0.74-0.75 respectively) indicating good internal reliability. Test-retest reliability has been shown to be moderate for both emotional overeating (0.52) and emotional under-eating (0.64) over a two week period (Wardle, Guthrie et al. 2001). The factor structure of the CEBQ has been replicated in different samples of children across many countries (Wardle, Guthrie et al. 2001; Carnell and Wardle 2007; Viana, Sinde et al. 2008; Svensson, Lundborg et al. 2011; Cao, Svensson et al. 2012; Sparks and Radnitz 2012; Mallan, Liu et al. 2013; Domoff, Miller et al. 2015). The full questionnaire can be found in Appendix 3.

The CEBQ-T is an amended version of the validated Child Eating Behaviour Questionnaire (CEBQ). The CEBQ-T was modified to be appropriate for toddlers. The majority of the items in the CEBQ and the CEBQ-T are identical. However, the Emotional Undereating and Desire to Drink scales from the original CEBQ were removed from the CEBQ-T as mothers reported their children did not to engage in these behaviors. Furthermore, the wording of EOE items was modified. Words describing the children's mood were changed to make them more age appropriate ('worried', 'annoyed' and 'anxious' were replaced with 'irritable', 'grumpy' and 'upset', respectively). Children needed to have a minimum of 2/3 items (CEBQ-T) or 3/4 items of the scales (CEBQ) completed to be included. The full CEBQ-T can be found in Appendix 4.

4.7 Measuring eating behaviour in infants – The Baby Eating Behaviour Questionnaire (BEBQ)

The Baby Eating Behaviour Questionnaire (BEBQ) is a modified version of the CEBQ to assess infant eating behaviour (Llewellyn, van Jaarsveld et al. 2011). The questionnaire consists of 18 items, rated by parents on a 5-point Likert scale. Principal Component Analyses revealed four distinct eating behaviours: Enjoyment of Food (EF), Food Responsiveness (FR), Slowness in Eating (SE) and Satiety Responsiveness (SR). The internal reliability of the constructs was good with Cronbach's alphas ranging from 0.73-0.81 (Llewellyn, van Jaarsveld et al. 2011). The validity of the questionnaire and the association between infant eating behaviour and weight has been confirmed in a second sample (Mallan, Daniels et al. 2014). Mean scores for each subscale were only calculated if a minimum of items were entered (2/3, 3/4 or 4/5). The full BEBQ can be found in the Appendix 5.

5 The twin method

5.1 The power of twin research

Over the past century the twin method has been used to investigate genetic and environmental contributions to variation in complex human traits. Researchers have been using this methodology to examine a wide spectrum of aspects of human life accumulating in a total of 17,804 investigated traits, spanning disease, to behaviour to opinion. Twin research is conducted worldwide and 14,558,903 twins are currently included in a multitude of studies (Polderman, Benyamin et al. 2015).

5.2 The underlying assumptions of the twin method

The twin method was formulised at the turn of the last century and its underlying assumptions remain today (Fisher 1919; Rende, Plomin et al. 1990). The twin method utilises the natural occurrence of identical, monozygotic (MZ) and non-identical, dizygotic (DZ) twins. As discussed in Chapter 1, comparing the resemblance between MZ twins and DZ twins on a measurable trait enables researchers to decompose the variation of the trait into genetic and environmental contributions. MZ twins are natural clones, sharing all of their genetic material, whereas DZ twins share on average 50% of their segregating genes, like any other regular siblings. Importantly, however, MZ and DZ twins are assumed to share their environments to a very similar extent (from the prenatal environment to later environmental factors). This being true, if MZ twins are more similar than DZ twins on the trait of interest, researchers assume a genetic contribution to trait variation because the only difference between the two types of twins is that MZs are twice as similar genetically, while both types of twins share their environments equally. Resemblance between MZ twins could reflect both their genetic relatedness as well as aspects of the shared environment, because they share 100% of both; but the extent to which they differ captures only environmental influences unique to each individual twin, as well as measurement error.

Hence comparison of MZ and DZ pairs allows for the variation of any given trait to be decomposed into three latent factors: (i) heritability or genetic effects (A), (ii) shared environmental effects (all factors that increase similarity between two twins in a pair, above and beyond genetic resemblance) (C), (iii) and non-shared environmental effects (factors that contribute to differences between the pairs), which also includes measurement error (E).

The following path diagram (Figure 5.1) illustrates the basic assumption of the twin method. Latent factors A, C and E are represented in circles and the measured phenotype (e.g. EOE) in rectangular

boxes for two twins in a pair. The double-headed arrows connect the twins, representing their relationship in accordance with their zygosity. MZ twins are genetically identical and so the correlation between the latent factor A is constrained to 1, whereas DZ twins only share on average half of their genes, so their correlation of genetic relatedness is fixed at 0.5. Regardless of their zygosity, both types of twins share their environments to the same extent, so the correlation for the shared environment (latent factor C) is fixed at 1 for both MZs and DZs. Because the non-shared environmental factors (E) contribute to differences between the twins, this latent factor is not correlated between them. All covariation between two twins in a pair must therefore be explained by latent factors A and C.

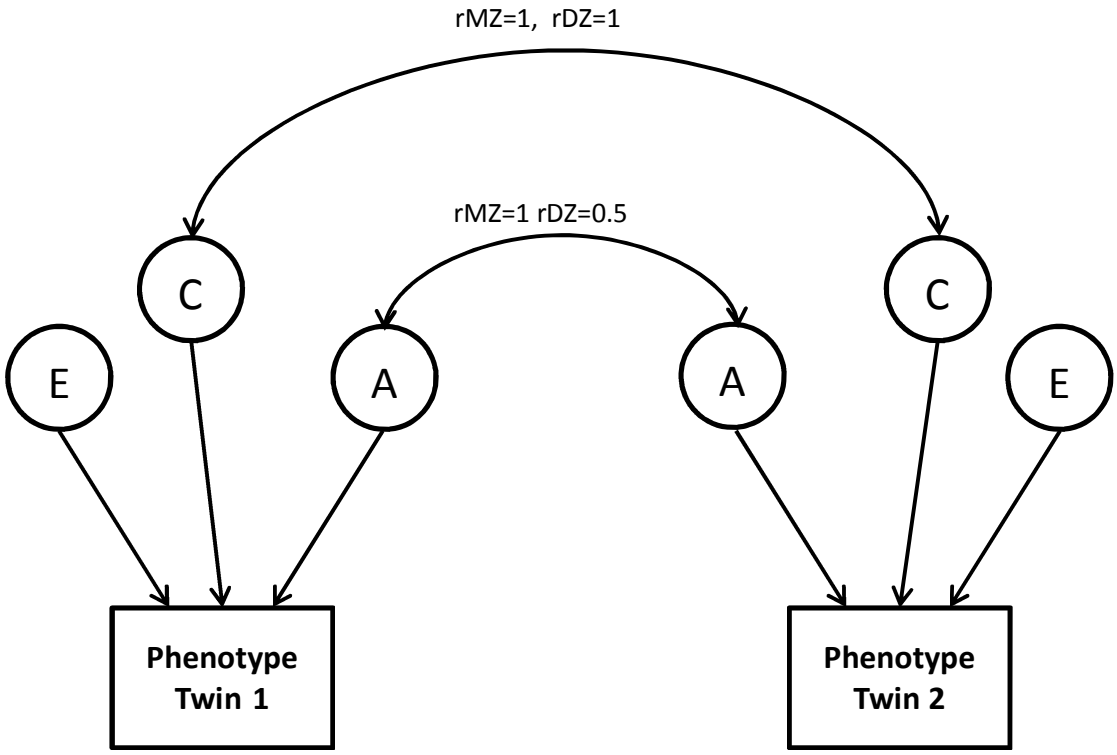


Figure 5.1 Path diagram representing the relationship between the latent factors A, C, and E for MZ and DZ twins

5.3 Estimating genetic and environmental contributions to variation in traits using twin correlations

5.3.1 Univariate twin model using correlations

The simplest way to calculate estimates for genetic and environmental contributions to variation in any given trait is to compare intraclass correlations (ICCs) for MZ and DZ pairs, using Falconer's Formula. The total variance (V) is decomposed into the three components A, C and E (V = A + C + E). The correlation between the MZ pairs reflects all genetic effects and all shared environmental effects: rMZ = 1A + 1C. For DZ twins the correlation reflects only half of the genetic effects (because they share approximately 50% of their genes), but all shared environmental effects: rDZ = 0.5A + 1C. Using these equations, the contributions of A, C and E to the total variance (V=1) can be calculated. Genetic effects (A), or heritability (h^2), are calculated by doubling the difference between the MZ and DZ correlations A = 2(rMZ - rDZ). Non-shared environmental factors are everything that does not contribute to MZ twin similarity: E = 1 - rMZ. Because the three variance components together amount to 1, the shared environmental contribution can be calculated from A and E: C = 1 – A + E. These calculated estimates give a rough indication of the relative contribution of genetic, shared and non-shared environmental contributions to variation in a trait. This univariate design is useful to estimate the contribution to variation in a single trait.

5.3.2 Bivariate twin model using correlations

This simple formula can be extended to two different traits in order to understand the extent to which common genetic and environmental influences underlie multiple traits – e.g. to understand if EOE and EUE share genes in common, or shared environmental factors in common. Bivariate designs are based on the same assumptions as the univariate model. Instead of estimating A, C and E for a single trait using correlations for that trait, correlations across two traits are used. Here, cross-twin cross-trait (CT-CT) correlations for MZ twins are compared with the CT-CT correlations for DZ twins to infer the relative contribution of A, C, and E to the overall phenotypic correlation between the two traits. For example, Twin 1's EOE is correlated with Twin 2's EUE, and Twin 2's EOE is correlated with Twin 1's EUE for both MZs and DZs. In line with the univariate model, if the MZ CT-CT correlation is higher than the DZ CT-CT correlation genetic effects are contributing to the phenotypic correlation between the 2 traits – i.e. there are common genetic influences underlying the two traits that contribute to their correlation. If there is no differences between the MZ and DZ CT-CT correlations, shared environmental influences underlie the correlation between the two traits. On the other hand, non-

significant CT-CT correlations indicate unique environmental effects as the most likely source for the phenotypic correlation between the two traits.

5.3.3 Longitudinal Twin Model

The same logic can be applied to investigate the longitudinal relationship of traits measured at multiple time points. Instead to of using cross-twin cross-trait correlations, cross-twin cross-time correlations are calculated. Here twin 1 at time 1 is correlated with twin 2 at time 2 and vice versa. As with simple ICCs, the pattern of MZ and DZ CT-CT correlations provides an indication of the extent to which continuing genetic or environmental influences drive the phenotypic association between two time points. Higher average CT-CT correlations for MZ pairs relative to DZ pairs indicates that common genetic factors at both ages contribute to the phenotypic association; similar CT-CT correlations for both types of twins indicates that common shared environmental effects at both ages are important in driving the phenotypic association. No significant CT-CT correlations indicate that unique environmental influences common to the trait at both time points underlie its prospective correlation.

5.4 Structural Equation Modelling (SEM)

Using twin correlations can provide only rough estimates of the relative contributions of A, C and E to variation in any given trait. Maximum Likelihood Structural Equation Modelling (MLSEM) uses variances and covariances instead of correlations, and provides more reliable estimates of A, C and E with 95% confidence intervals and goodness-of-fit statistics.

MLSEM is carried out in OpenMx software version 32, a software package designed for R (Virginia Commonwealth University, Richmond, VA). A number of fit statistics are available, including the Likelihood Ratio test, Aikaike's Information Criterion (AIC) and the Bayesian Information Criterion (BIC). BIC was the preferred goodness-of-fit statistic for the analyses in this thesis because it takes account of the sample size and is less conservative than the LRT and AIC with large datasets like Gemini (Posada and Buckley 2004). The model with the lowest BIC value is the model that is considered to fit the best.

First a saturated model is fitted to the data, with no parameter constraints (i.e. estimating only means, variances and covariances for MZs and DZs), to provide fit statistics against which to test the goodness of fit of the ACE model, and subsequent submodels. Then a full ACE model is fitted. For the univariate analyses more parsimonious sub-models are then tested for goodness-of-fit against the full ACE model; sub-models drop A, C, and A and C together (E is never dropped from the model because it

includes measurement error). The model producing the lowest BIC is then selected. For longitudinal analyses, a full ACE model is fitted first and compared to a saturated model for goodness-of-fit. Non-significant parameters are then dropped to identify the most parsimonious model with the lowest BIC value.

Like the CT-CT correlations, a longitudinal model provides useful information about the extent to which the genetic, shared environmental and unique environmental influences underlying a trait at time point 1 are the same as those at time point 2, denoted by the aetiological correlations: the genetic [r_A], shared environmental [r_C], and unique environmental [r_E] correlations. A high r_A would indicate that the majority of the genetic effects at time point 1 persist at time point 2, whereas a low r_A would indicate that genetic factors are largely unique to each age. The longitudinal model also quantifies the extent to which continuing genetic and environmental influences explain the longitudinal phenotypic correlation from time point 1 to time point 2 (denoted as bivariate A, C and E). That is, the bivariate estimates explain whether stability of a trait from time point 1 to time point 2 is largely due to the same genes or the same environmental factors influencing the trait at both ages. Bivariate estimates and the aetiological correlations (r_A, r_C, and r_E) are independent of the univariate A, C and E contributions at individual time points – e.g. it is possible for a trait to be highly heritable at both time points, and correlated over time, with few genetic effects in common at either age (low bivariate A), and the longitudinal association being driven entirely by common environmental effects (low r_A, and high r_C or r_E) (Posthuma, Beem et al. 2003).

5.5 Limitations of the twin method

The twin model has been shown to be a consistent and reliable research methodology. However, the twin method has its limitations. These are summarised in the sections below.

5.5.1 Representativeness of twins

In order to interpret findings from twin research, twins must be representative of the general population, i.e. singletons. Compared with age-matched singletons twins have been shown not to differ on various physical and behavioural traits, including: bone mineral density, blood pressure, alcohol and tobacco consumption (Andrew, Hart et al. 2001), personality traits (Johnson, Krueger et al. 2002), and motor development (Wilson and Harpring 1972). However twins are born earlier and have a lower birth weight, even taking into account their size at birth relative to their gestational age (van Dommelen, de Gunst et al. 2008). They experience ‘catch up’ growth after birth, reaching a

similar size to singletons at around 2.5 years of age (Bleker, Breur et al. 1979; Wilson 1979; van Dommelen, de Gunst et al. 2008). However, older twins mostly do not differ from singletons, and twin cohorts are therefore deemed representative.

5.5.2 Violation of the equal environments assumption (EEA)

The EEA states that environmental exposures influencing the variation of a trait are unrelated to the zygosity of the twin pairs – i.e. that MZs and DZs share their environments to the same extent. A violation of the EEA could lead to an overestimation of the genetic contribution to variation if MZs, in fact, share their environments more closely than DZs. This is because the higher MZ correlation would reflect both increased shared environmental influences, as well as increased similarity in genetic relatedness, compared to the DZ correlation, rather than just increased genetic relatedness. Because heritability is estimated by doubling the difference between the MZ and DZ correlation, a higher MZ correlation due to greater environmental similarity would be masked as heritability.

Before birth MZ and DZ twins share the same prenatal environment and are exposed to the same environmental factors influencing the pregnant mother (Rijsdijk and Sham 2002). Furthermore, both MZ and DZ pairs tend to grow up in the same family from birth until they leave home. However, the fact MZ twins look identical and are often perceived as more similar (by virtue of the fact that they are more similar on all genetically-determined traits), has given rise to the claim that they might be treated more similarly by their parents in comparison to DZ twins (Felson 2014).

The EEA has been widely debated and still remains controversial (Fosse, Joseph et al. 2015). It has been pointed out as a fundamental flaw of the twin method, and poses a challenge to the validity of twin research (Joseph 2013). However multiple attempts to test the potential violation of the EEA have been conducted (Goodman and Stevenson 1989; Morris-Yates, Andrews et al. 1990; Kendler, Neale et al. 1993; Hettema, Neale et al. 1995; Xian, Scherrer et al. 2000; Borkenau, Riemann et al. 2002; Cronk, Slutske et al. 2002; Conley, Rauscher et al. 2013; Felson 2014; LoParo and Waldman 2014). These are discussed in more detail in the following section.

5.5.3 Accounting for physical resemblance, environmental exposure and social contact

One way to test the validity of the EEA is to see if MZ twins are treated more similarly due to their physical resemblance. In order to adhere to the EEA these similarities should not be associated with the intraclass correlations of MZ and DZ twins. Multiple studies have investigated this notion, addressing physical similarity. Even though studies found evidence that MZ twins do look more

similar, there were no associations found between physical resemblance and correlations on various traits such as eating attitudes, personality traits, intelligence and reading skills. In other words, no violations of the EEA were detected accounting for physical resemblance in MZ twins (Matheny, Wilson et al. 1976; Plomin, Willerman et al. 1976; Hettema, Neale et al. 1995; Klump, Holly et al. 2000).

Other studies have examined if MZ twins are exposed to more similar environmental exposures than DZ twins. To do so twins rated their upbringing and other environmental exposures retrospectively. MZ twins were indeed found to experience more similar environments but no associations between similarity in environmental exposure and correlations on various traits such as anxiety and depression (Morris-Yates, Andrews et al. 1990), binge eating disorder (Bulik, Sullivan et al. 1998) or externalizing disorders (LoParo and Waldman 2014) were found. These findings support the validity of the EEA, however the retrospective nature of the data collection (e.g. childhood memories), could have influenced the accuracy of measurement of environmental exposure.

Another factor that could potentially lead to violation of the EEA, is the idea that adult MZ twins have a stronger personal relationship with their co-twin than adult DZ twins, and might therefore be more similar if increased social contact is maintained. Several studies have investigated if the degree of social contact between the twin pairs is associated with the correlation on behavioural traits. No associations were found between increased social contact and correlations on personality traits such as neuroticism and extraversion (Rose, Koskenvuo et al. 1988; Kaprio, Koskenvuo et al. 1990), substance abuse disorder (LaBuda, Svikis et al. 1997) or rates of physical activity (Eriksson, Rasmussen et al. 2006).

Further evidence that a close personal relationship between MZ twins does not contribute to greater similarity than DZ twins comes from studies using the identical twins raised apart design. Here correlations between identical twins raised together are compared with those of identical twins reared apart. Research has found that MZ twins correlate highly on anthropometrics (e.g. BMI and waist circumference) (Zhou, Gao et al. 2015), IQ (Bouchard, Lykken et al. 1990) and personality traits such as impulsiveness (Coccaro, Bergeman et al. 1993), regardless of whether they were raised together or apart.

5.5.4 The 'misclassified zygosity' design

Another way to test the EEA is the 'misclassified zygosity' design. Sometimes twins are misinformed about their zygosity, or simply believe they are non-identical even though they are, in fact, identical. The 'misclassified zygosity' design exploits this occurrence to test the EEA by comparing the correlations of a trait for MZ pairs who correctly believe themselves to be MZs, and MZ pairs who have misclassified themselves as DZs. Matching correlations across both types of MZs are seen as support for the EEA. Early research using this design supported the EEA insofar as identical twins were found to correlate to the same extent on personality traits and cognitive ability, regardless of their believed zygosity (Scarr and Carter-Saltzman 1979). Since then the misclassified zygosity design has been used to provide support for the validity of the EEA in relation to a range of other traits, including: hyperactivity, major depression, generalized anxiety disorder, phobia, bulimia, post-traumatic stress disorder, alcohol and nicotine dependence as well as dieting patterns (Goodman and Stevenson 1989; Kendler, Neale et al. 1993; Kendler, Neale et al. 1994; Xian, Scherrer et al. 2000; Cronk, Slutske et al. 2002; Gunderson, Tsai et al. 2006; Conley, Rauscher et al. 2013). A recent review concluded that the EEA is valid for most traits, and if violated would only result in a minor inflation of heritability, of no more than 10% (Felson 2014).

5.5.5 Potential for rater bias in relation to zygosity

Twin studies with samples of children often rely on parent rated measures. One of the criticisms of parent-rated measures in twin research is that parents' ratings of their twins' behaviour might be biased by their perception of their twins' zygosity. For example, parents might be inclined to rate their twins more similarly if they believe them to be identical. On the other hand parents who believe their twins to be non-identical might rate them more differently. This potential parental bias would result in an inflated difference between MZ and DZ correlations, and therefore an overestimation of genetic effects (which are estimated by doubling the difference between the MZ and DZ correlations).

6 Study 1. Testing for parental reporting bias in relation to perceived zygosity for eating behaviours in children

6.1 Background

As discussed in detail in Chapter 5, the twin method is based on the comparison of correlations of identical and non-identical twins. One issue is that when analysing infant and childhood samples, the collected data is often, by necessity, parent rated. This provides an opportunity for parental bias to occur. As described in Section 5.5.5., a particular bias of concern for twin studies is rater bias in relation to perceived zygosity. The 'misclassified zygosity' design, comparing correctly classified and misclassified sets of identical twins, can be used to test for parental bias in twin studies. This has never before been applied to test for bias related to zygosity status in parental reports of their twins' behaviour.

6.2 Aims

The aim of this study was to use a 'misclassified zygosity' design to test for parental bias in relation to zygosity, in parent-reported measures of eating behaviour in Gemini during infancy and toddlerhood.

6.3 Methods

6.3.1 Measures

The zygosity of the twins was established using the zygosity questionnaire and DNA, as described in Chapter 4. These analyses only used same-sex pairs of twins; opposite-sex pairs (n=816, 33.3%) and pairs of unknown zygosity (n=37, 1.5%) were excluded. To establish which parents correctly classified or misclassified the zygosity of their twins, parents answered the question: "Do you think your twins are identical?" when the twins were on average two years old, and their responses were compared with our zygosity classification (using both the validated zygosity questionnaire and DNA). Data from the parent-rated Baby Eating Behaviour Questionnaire (BEBQ) and Child Eating Behaviour Questionnaire -Toddler version (CEBQ-T) were used to calculate intraclass correlations (ICCs) for the different scales.

6.3.2 Analyses

Four groups were created from the parental classifications of zygosity, and zygosity established using the questionnaire and DNA: (i) MZC, MZ pairs correctly classified as MZ by parents; (ii) MZI, MZ pairs misclassified as DZs by parents; (iii) DZC, DZ pairs correctly classified as DZ by parents; (iv) DZI, DZ pairs misclassified as MZs by parents.

Intraclass correlations with 95% confidence intervals for the four eating behaviours measured by the BEBQ at eight months and for the six eating behaviours measured by the CEBQ-T at 16 months were calculated for correctly classified and misclassified zygosity pairs. Scores on the BEBQ and CEBQ-T were adjusted for age, sex and gestational age using regression analyses. Analyses were conducted using SPSS 22 for windows.

6.4 Results

6.4.1 Zygosity groups

29.4% (220/749) of parents of MZ twins mistakenly believed them to be dizygotic. Only six parents of same-sex DZ pairs mistakenly classified them as MZs (0.75% of parents of same sex DZs, 6/800); because of the small sample size for these pairs the 95% confidence intervals were wide and reliable ICCs could not be calculated. We therefore only report the results for three groups: MZC, MZI, and DZC. All percentages and numbers of twin pairs included in this analysis are shown in Table 6.1.

Table 6.1 Numbers and percentages of twin pairs for the different zygosity groups

Sample of same sex twin pairs (excluding unknown zygosity)	**Frequency (n)**	**Percent (%)**
Total	1549	
MZ	749	48.4
DZ	800	51.6
Zygosity groups according to parents' beliefs of zygosity and zygosity derived from questionnaire and DNA data		
Total	1528**	100
MZC*	511	33.4
MZI*	220	14.4
DZC*	791	51.8
DZI*	6	0.4

* Abbreviations: MZ, Monozygotic; DZ, Dizygotic; MZC, MZ pairs correctly classified as MZ by parents; MZI, MZ pairs misclassified as DZs by parents; DZC, DZ pairs correctly classified as DZ by parents; DZI, DZ pairs misclassified as MZs by parents

** This number is less than the total sample of classifiable (from questionnaire data and/or DNA) same-sex twins, because of the 1549 MZs and same-sex DZs, 21 families did not respond to the

question “Do you think your twins are identical or non-identical?” and so could not be included in these analyses.

6.4.2 Intraclass Correlations

6.4.2.1 *Baby Eating Behaviour Questionnaire*

Overall there was no difference in magnitude between the size of the ICCs for correctly and misclassified identical twins for any of the four eating behaviors. For SR, EF and SE the 95% confidence intervals overlapped, indicating that the ICCs were not significantly different for MZC and MZI. The 95% confidence intervals did not overlap for the ICCs for FR, however the difference in magnitude was very small (MZC, 0.89; MZI, 0.82) and the large sample size ensured that the 95% confidence intervals were narrow, such that trivial differences were significant. Importantly, the ICCs for the DZC group were substantially smaller than those for the MZI group for all four eating behaviors, and none of the 95% confidence intervals overlapped.

6.4.2.2 *Child Eating Behaviour Questionnaire*

A similar pattern of results was found for eating behaviors measured by the CEBQ at 16 months. For each of the five eating behaviors the magnitude of the ICCs for MZC and MZI was similar. For EF and FF there was no significant difference between MZC and MZI, indicated by the overlapping 95% confidence intervals. For SR, FR and EOE the 95% confidence intervals did not overlap for the MZC and MZI groups. However, the 95% confidence intervals were narrow because of the large sample size, and the differences were very small (EOE Δ =0.02; FR Δ = 0.04; SR Δ = 0.05) and in no consistent direction (the MZC ICC was slightly higher for FR but slightly lower for SR and EOE). Again, importantly the ICCs for the DZC group were substantially smaller than the MZI ICCs for each of the five eating behaviors, and none of the 95% confidence intervals overlapped. All ICCs for the different zygosity groups and eating behaviors are presented in Table 6.2.

Table 6.2 Intraclass Correlations for four appetitive traits measured at three months (BEBQ) and for six appetitive traits measured at 15 months (CEBQ-T); for correctly and misclassified zygosity

BEBQ 3 months	**MZC***	**MZI***	**DZC***
SR*	0.84	0.8	0.51
95% CI	0.81-0.86	0.75-0.84	0.45-0.56
n (pairs)	512	215	772
FR*	0.89	0.82	0.6
95% CI	0.87-0.91	0.77-0.86	0.55-0.64
n (pairs)	500	215	768
EF*	0.8	0.8	0.47
95% CI	0.76-0.83	0.75-0.85	0.14-0.52
n (pairs)	511	212	769
SE*	0.82	0.82	0.4
95% CI	0.79-0.85	0.77-0.86	0.39-0.46
n (pairs)	502	216	772
CEBQ 15 months	**MZC***	**MZI***	**DZC***
SR*	0.87	0.93	0.65
95% CI	0.85-0.89	0.91-0.95	0.60-0.69
n (pairs)	413	187	634
FR*	0.91	0.95	0.71
95% CI	0.89-0.93	0.94-0.96	0.67-0.75
n (pairs)	413	186	630
EF*	0.89	0.88	0.6
95% CI	0.87-0.91	0.84-0.91	0.55-0.65

n (pairs)	413	187	629
FF*	0.87	0.81	0.55
95% CI	0.85-0.89	0.75-0.85	0.49-0.6
n (pairs)	415	187	632
EOE*	0.97	0.99	0.92
95% CI	0.96-0.97	0.98-0.99	0.9-0.93
n (pairs)	411	187	630
SE*	0.85	0.87	0.43
95% CI	0.82-0.97	0.82-0.89	0.36-0.49
n (pairs)	413	187	629

* Abbreviations; MZ, Monozygotic; DZ, Dizygotic; SR, Satiety Responsiveness; FR, Food Responsiveness; EF, Enjoyment of Food; FF, Food Fussiness; EOE, Emotional Overeating; SE, Slowness of Eating; MZC, MZ pairs correctly classified as MZ by parents; MZI, MZ pairs misclassified as DZs by parents; DZC, DZ pairs correctly classified as DZ by parents

6.5 Discussion

We used the 'misclassification of zygosity' design in a novel approach to test for parental bias in reporting of infant and child eating behavior. We showed for the first time that parents who misclassified their MZs as DZs nevertheless scored them as similarly as the parents who correctly classified their MZs as MZs, on a range of eating behaviors during both infancy and toddlerhood. Samples of misclassified zygosity were used to test for parental bias and the validity of the twin method. To do so intraclass correlations were compared for misclassified and correctly classified MZ pairs for a range of eating behaviors, measured by widely used parent-report questionnaires for infants (the BEBQ) and toddlers (the CEBQ-T).

The results showed that the magnitude of the intraclass correlations was very similar across both correctly and misclassified identical twins. In addition, the intraclass correlations for the correctly classified DZs were markedly smaller than those of the incorrectly classified MZs, and none of the 95% confidence intervals overlapped across the two groups. These results indicate that parents' perceptions of their twins' zygosity did not bias their scoring of their eating behaviors, insofar as they

did not score their MZ twins less similarly if they mistakenly believed them to be DZ. The problem of parental rater bias is often raised in research with infants and children. These outcomes suggest that no parental bias was found in relation to zygosity status when using parental questionnaires of eating behavior, and supports the validity of the twin method for establishing the genetic and environmental influences on eating behaviors in infants and toddlers.

As previously reported, parents can be misinformed about the zygosity of their children (Ooki, Yokoyama et al. 2004). In this sample, of 749 MZ twins, 220 (29.4%) were misclassified as DZ. Previous research suggests that parental misclassification of MZs as DZs often stems from false information given by health professionals (van Jaarsveld, Llewellyn et al. 2012). Many health professionals classify twin pairs as non-identical if a prenatal scan shows that they are dichorionic (each has their own placenta), regardless the fact that approximately one third of MZ twin pairs develop with separate placentas (Hall 2003). Knowledge gaps of obstetricians and gynecologists in twin prenatal development is suggested to be the cause of the misinformation (Cleary-Goldman, Morgan et al. 2005). Using reliable measures of zygosity determination in same-sex twins is crucial for twin research. Additionally, zygosity classifications are important for medical reasons, such as prenatal diagnosis of genetic disease or disorders and transplant compatibility, as well as the identity and social development of the children (Stewart 2000; Hall 2003).

Limitations

In the current sample only a small number of same sex DZ pairs were misclassified as MZ (N=6). Due to the tiny sample, intraclass correlations were not significant and had wide 95% confidence intervals, making them difficult to interpret. Future studies using the misclassified zygosity design would benefit from increased sample sizes to include more misclassified DZs. Larger samples would enable researchers to make comparisons between correctly classified and misclassified DZ twins, to provide more evidence in support of the validity of parental reports for the twin method.

This study only assessed parental bias in relation to eating behavior in infancy and toddlerhood. Additional studies using a similar design could investigate bias on other parent rated child behaviors, such as physical activity and personality. It would also be useful to understand if parental bias starts to emerge as children mature and naturally become more different from another. Future studies using the misclassified zygosity design assessing parental bias in school children would be useful.

Conclusion

A potential flaw in the twin method is parental bias in reports of twin behavior, related to perceived zygosity. The outcomes of this study suggest that there was no parental bias related to zygosity in the Gemini twin cohort when parents reported on a range of infant and child eating behaviors.

7 Study 2: Genetic and environmental contributions to individual differences in EOE at 16 months to 5 years and trait stability

7.1 Introduction

As described in Chapters 1 and 2, emotional overeating has been associated with increased weight in adults and children. There is a paucity of twin studies examining the aetiology of emotional overeating even in adults (n=4 studies). As Chapter 2 highlighted, adult twin studies have tended to indicate moderate contributions from genetic influences, with the majority of variance explained by non-shared environmental influences. So far no twin studies have investigated the relative genetic and environmental contributions to emotional overeating in children. Previous paediatric studies of other eating behaviours such as satiety responsiveness and food responsiveness have reported substantial heritability in infancy (Llewellyn, van Jaarsveld et al. 2010) and childhood (aged 8-11 years) (Carnell, Haworth et al. 2008), although theories of EOE suggest that it is largely learned.

7.2 Aims

The aim of this study was to examine the environmental and genetic contributions to individual differences in emotional overeating at 16 months and 5 years, and to quantify the extent to which common genetic or environmental influences contribute to trait stability over the same developmental period.

7.3 Methods

7.3.1 Measures

Emotional Overeating was measured with the CEBQ-T when twins were 16 months old and the standard CEBQ when they were 5 years old. Both questionnaires are described in Chapter 3. Participants needed to have a minimum of 2/3 items of the scales completed to be included.

7.3.2 Analyses

Because twins share their age and gestational age exactly (and sex for same-sex pairs), it is standard practice to regress scores on gestational age, age at the time of measurement, and sex prior to twin analyses to ensure these factors do not inflate the shared environmental effect. All twin analyses were performed on the regressed EOE scores. Pearson's correlation coefficient was used to establish the association between EOE at 16 months and 5 years.

7.3.2.1 Twin Analyses

The basis of the twin method is described in detail in Chapter 4. In this study genetic and environmental contributions to variation in EOE were estimated using two methods: comparisons of intraclass correlations (ICCs) and maximum likelihood structural equation modelling (MLSEM).

7.4.2.2 Intraclass correlations

ICCs for EOE were calculated and compared for MZs and DZs at 16 months and at 5 years. As described in Chapter 4, the pattern of resemblance provides an indication of the relative importance of genetic and environmental influences on EOE at each age. Cross-twin cross-time (CT-CT) correlations provide an indication of the contribution of continuing genetic and environmental influences to the longitudinal phenotypic association (the stability of EOE from 16 months to 5 years). ICCs and CT-CT correlations were calculated using SPSS Version 21 and OpenMx software version 32 (Virginia Commonwealth University, Richmond, VA).

7.4.2.3 Maximum likelihood structural equation modelling

Maximum Likelihood structural equation modelling (MLSEM) was used to provide reliable parameter estimates of genetic effects (A), shared environmental effects (C) and unique environmental effects (E) with 95% confidence intervals and goodness-of-fit statistics. Univariate models were run to provide estimates of A, C and E at 16 months and 5 years. A longitudinal model provided information about the extent to which the genetic, shared environmental and unique environmental influences underlying EOE at 16 months were the same as those at 5 years, denoted by the genetic [r_A], shared environmental [r_C], and unique environmental [r_E] correlations. The longitudinal model also quantified the extent to which continuing genetic and environmental influences explained the longitudinal phenotypic correlation from 16 months to 5 years (denoted as bivariate A, C and E).

7.4.3 Results

EOE scores were available for 3774 children at 16 months and 1986 children at 5 years, with a combined sample for the analysis of 3784 children who had data at either 16 months, 5 years or both ages (MLSEM is able to include participants who have missing data at one time point). Participants with unknown zygosity were excluded from analyses (n= 37). The descriptive statistics for the analysis sample are shown in Table 7.1. There were some differences between families who provided data at *both* 16 months and 5 years, with those who only provided data at 16 months. Mothers of children who remained in the study were more educated, older and had a lower BMI at baseline. However, the children included in these analyses did not differ from the full sample in terms of zygosity, sex,

gestational age or birth weight. EOE at 16 months was significantly associated with EOE at 5 years of age (r=0.248, p<0.001), such that toddlers who were prone to eating more in response to negative emotions tended to do this as young children as well.

Table 7.1 Descriptive statistics for analysis sample (n=3784; 1892 twin pairs)

Twins	N (%)
Total	1892
Zygosity	
MZ	613 (32.4)
DZ	1279 (67.6)
Sex	
Males	1860 (49.2)
Females	1924 (50.8)
	Mean (SD)
Gestational age (weeks)	36.21 (2.47)
Weight at birth (kg)	2.46 (0.54)
Age at 16 months (months)	15.82 (1.15)
Emotional Overeating at 16 months	1.64 (0.59)
Age at 5 years (years)	5.15 (0.13)
Emotional Overeating at 5 years	1.38 (0.48)

7.4.4 Univariate analyses

The intraclass correlations for the twin pairs at 16 months and 5 years are shown in Table 7.2. At both ages the MZ and DZ correlations were high and similar for both types of twins. This suggested a low contribution from genes and a strong contribution from the shared environment to variation in EOE.

MLSEM was used to calculate the univariate estimates for 16 months and 5 years (Table 7.3). In line with the patterns observed for the ICCs, at both 16 months and 5 years, genetic effects were very small but significant (10% and 4% respectively). The majority of variance in EOE was explained by shared environmental effects at both 16 months (88%) and 5 years (93%). The variance explained by the unique environment at each age was small. A full ACE model was found to fit the data best i.e. none of the parameters could be dropped, without a worsening of fit.

Table 7.2 Intraclass Correlation (ICCs) and Cross-Twin Cross-Time correlations (with 95% Confidence Intervals) for Emotional Overeating scores measured at 16 months and 5 years

	MZ[1]	DZ[1]
16 months	0.97	0.92
ICCs[1] (95% CI[1])	(0.97-0.98)	(0.92-0.93)
5 years	0.97	0.95
ICCs (95% CI)	(0.97-0.98)	(0.94-0.96)
CT CT[12] (95% CI)	0.25	0.25
	(0.19-0.30)	(0.20-0.31)

[1]Abbreviations: MZ: Monozygotic; DZ: Dizygotic; ICCs: Intraclass Correlation, CI: Confidence Intervals, CT CT, cross-twin cross-time intraclass correlation

[2] MLSEM provides an average correlation between the two twins across the two time points. The ratio of the MZ and DZ CT CT gives an indication of genetic and environmental aetiology.

7.4.5 Longitudinal analyses

The CT-CT correlation demonstrated similar patterns to the simple ICCs. This suggested that continuing shared environmental influences largely explain the correlation from 16 months to 5 years, and that there are few continuing genetic influences that contribute to stability in EOE from toddlerhood to early childhood. CT-CT correlations are presented in Table 7.2.

The A, C and E estimates derived from the longitudinal MLSEM were the same as those from the univariate analyses. The full ACE longitudinal model was the best fitting model, according to the BIC statistic. Two submodels were also tested – one that dropped the bivariate E estimate, and one that dropped both the bivariate A and E estimates – but the change in the BIC value from the full ACE model to either of the submodels was not large enough to indicate that either of the submodels provided a better and more parsimonious fit to the data. The results from the full ACE model are therefore presented. A full list of fit statistics are presented in Table 7.4. A path diagram of the full longitudinal ACE model is presented in Figure 7.1.

Table 7.3 Estimates for A, C and E (95% Confidence Intervals) and fit statistics for the full ACE model and the nested submodels (CE, AE and E) derived from the Maximum Likelihood Structural Equation Modelling

Emotional Overeating	Model	Genetic effect (A)	Shared environmental effect (C)	Non-shared environmental effect (E)	-2LL[3]	df[3]	BIC[3]	Δ BIC[3]	change in chi-square (df)	p-value
16 months	Sat[3]				6427.265	3764	-10981.811			
n= 3832	**ACE[1]**	**0.098 (0.08 -0.11)**	**0.88 (0.86 -0.89)**	**0.02 (0.024 -0.031)**	**6433.163**	**3769**	**-10997.719**	**15.908**	**5.898 (5)**	**0.41**
	CE[2]	0	0.94 (0.93-0.94)	0.06 (0.05 -0.07)	6622.852	3770	-10906.646	-91.073	189.689 (1)	<0.01
	AE[2]	0.97 (0.96 -0.97)	0	0.03 (0.03-0.04)	7758.528	3770	-10338.808	-658.911	1325.365 (1)	<0.01
	E[2]	0	0	1	10681.818	3771	*	*	*	<0.01
5 years n= 1996	Sat[3]				3103.066	1976	-5266.389			
	ACE[1]	**0.04 (0.03-0.06)**	**0.93 (0.91 -0.94)**	**0.03 (0.02 -0.03)**	**3107.62**	**1981**	**-5281.363**	**14.974**	**4.554 (5)**	**0.6**
	CE[2]	0	0.96 (0.95-0.96)	0.04 (0.04 -0.05)	3143.922	1982	-5266.663	-14.7	36.302 (1)	<0.01
	AE[2]	0.97 (0.96-0.97)	0	0.03 (0.03-0.04)	4044.803	1982	-4816.223	-465.24	1297.183 (1)	<0.01
	E[2]	0	0	1	5631.227	1983	-4026.461	-1254.906	2523.607 (2)	<0.01

[1] Full ACE Model nested within the saturated model

[2] Submodels nested within the full ACE model, one factor is dropped after the other. All submodels compared against the full ACE model with the best fitting model highlighted in bold

[3]Abbreviations; - 2LL: -2 times log-likelihood of data; Δ-2LL: difference in 2 times log-likelihood; df: degrees of freedom, BIC: Bayesian information criterion; ΔBIC: change in BIC

There was a significant positive shared environmental correlation (r_C=0.29; 95% CI: 0.23-0.35), indicating that approximately one third of the shared environmental factors that influence a child's tendency to emotionally overeat at 15 months, persist to 5 years exerting the same effects. There was also a significant negative genetic correlation between the two time points (r_A= -0.26; 95% CI: -0.45- -0.08). However, because the genetic components of variance at both ages (especially at age 5 years, 4%) were very small, the genetic correlation is unreliable and difficult to interpret. Lastly, the non-shared environmental correlation was non non-significant (r_E = 0.03; 95% CI: -0.11 - 0.17).

The bivariate estimates quantified the contribution of common genetic and environmental factors to the longitudinal association between EOE at 16 months and 5 years. These suggested that the longitudinal association was completely driven by shared environmental effects (bivariate C: 1.07; 95% CI: 1.03-1.11). Bivariate A was very small (bivariate A: -0.07; 95% CI:-0.12 - -0.02) and bivariate E was found to be non-significant (bivariate E: 0.00; 95% CI:-0.01-0.02). These results made sense in the light of the fact that shared environmental factors were largely driving variation in EOE at both ages.

Table 7.4 Fit statistics for longitudinal models for emotional overeating at 16 months and 5 years

Fit Statistics						
Model	**-2LL[1]**	**df[1]**	**BIC[1]**	**ΔBIC[1]**	**Δ -2LL (df)**	**p-value**
Sat[1]	9448.452	5732	-16900.861			
ACE[1]	9456.383	5743	-16938.395	37.529	7.931 (11)	0.72
Submodel 1[2]	9464.649	5744	-16938.035	-0.36	8.267 (1)	<0.01
Submodel 2[3]	9468.468	5745	-16939.898	1.503	12.085 (2)	<0.01

[1] Abbreviations:, - 2LL: -2 times log-likelihood of data, Δ-2LL: difference in 2 times log-likelihood; df: degrees of freedom, BIC: Bayesian Information Criterion, Δ BIC: change in Bayesian Information, Sat: Saturated model, ACE: Full model including all factors.

[2] Submodel 1: In this submodel Bivariate A and the additive genetic correlation (r_A) were dropped. Submodel 1 is nested in and compared against the full ACE model.

[3] Submodel 2: In this Submodel Bivariate A, Bivariate E, the additive genetic correlation (rA) and the non-shared environmental correlation (rE) were dropped. Submodel 2 is nested in and compared against the full ACE model.

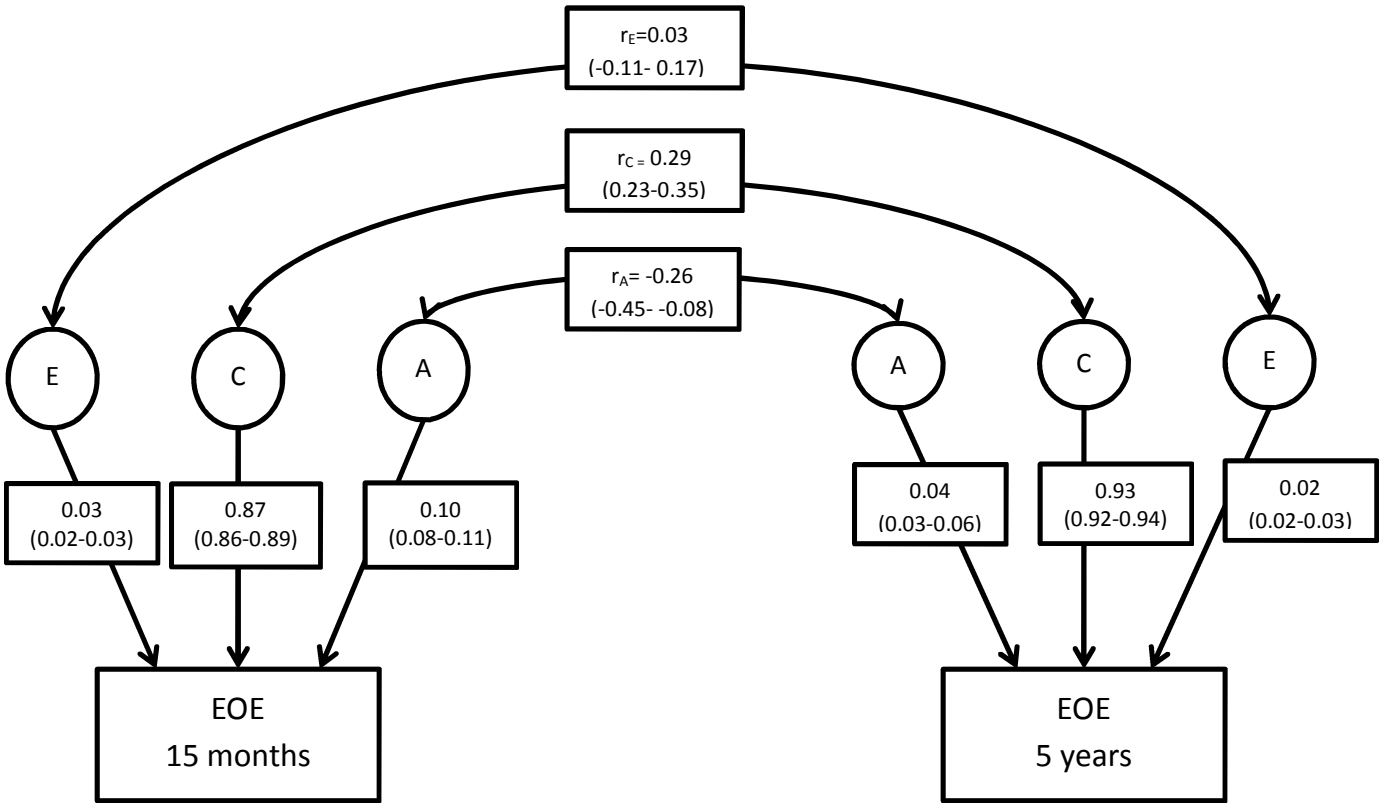


Figure 7.1 Full ACE Model Path Diagram.

The rectangular boxes represent the measured phenotype (EOE) using the Child Eating Behaviour Questionnaire at 15 months and 5 years. The circles indicate the latent factors of additive genetic effects (A), shared environmental effects (C) and non-shared environmental effects (E). The straight single-headed arrows reflect casual pathways with the variance explained by each latent factor (including 95% confidence intervals). The curved double headed arrows show the common genetic, shared environmental and unique environmental factors across the two time points that account for the longitudinal association. The aetiological correlations (r_A, r_C and r_E) are shown on the curved double-headed arrows on the bottom (the proportion of genetic (r_A), shared environmental (r_C) and unique environmental (r_E) influences that are common across the two ages) This figure illustrates the longitudinal model including all factors. To find the best fitting model non-significant factors were dropped from the model to find the most parsimonious and best fitting model.

7.5 Discussion

Summary of findings

This is the first pediatric study to investigate genetic and environmental contributions to the development of EOE, tracking children from toddlerhood (16 months) to early childhood (5 years). The results suggested a substantial effect of the shared environment on EOE in early life. However, it was somewhat surprising to observe that additive genetic effects contributed so little to this trait at

either age (10% and 4% respectively at 15 months and 5 years). These findings contrast with the high heritability estimates observed for other eating behaviours – Satiety Responsiveness (63%) and Enjoyment of Food (75%) – measured in 10 year-old children (Carnell, Haworth et al. 2008). They also contrast with the high heritability of four eating behaviours measured in Gemini at 3 months of age: Satiety Responsiveness (72%); Slowness of Eating (84%); Food Responsiveness (59%); and Enjoyment of food (54%) (Llewellyn, van Jaarsveld et al. 2010).

Evidence for the importance of the shared environment in shaping individual differences in this trait during both toddlerhood (88%) and early childhood (93%) also contrasts with previous studies of EOE in adults. These studies found no role of the shared environment, and a moderate contribution from genetic influences (Tholin, Rasmussen et al. 2005; Keskitalo, Tuorila et al. 2008; Sung, Lee et al. 2010). However, heritability estimates are known to vary by age, and previous studies of EOE have only used adult samples. It is perhaps unsurprising that for young children the shared environment plays an important role in shaping the development of this trait as parents are extremely involved in their child's eating behaviour. Another explanation for the difference between the studies could be that EOE in childhood and adulthood are different constructs. Children may engage in EOE as a strategy to regulate negative emotions, whereas overweight adults who usually exert some restraint may engage in EOE because of an inability to restrain their intake under conditions of stress (Adam and Epel 2007). Although, the Psychosomatic Theory of emotional overeating would indicate that it is indeed learned in early childhood.

We observed that EOE showed moderate stability from 16 months to 5 years (r=0.25); and the longitudinal association could be explained largely by continuing shared environmental influences from toddlerhood to early childhood. Almost one third of the shared environmental factors that influence EOE at 16 months continue to influence this trait at 5 years (rC=0.27); indicating that even though continuing aspects of the shared environment account for the stability of EOE (i.e. they largely explain the longitudinal association), many new shared environmental influences come into play at 5 years. There were no significant genetic or unique environmental effects that continued from toddlerhood to early childhood.

Implications and future directions

One possible explanation for observing such high influences from the shared environment, and such small genetic effects in this study is 'gene-environment correlation'. In order for genetic influences to

play out, individuals need the agency to make independent choices, so that they can 'act out' their genetically predisposed traits. The young age of the sample could explain the high impact of shared environments, as toddlers and children have limited access to food to regulate their emotions, and are unable to freely engage in emotional overeating. Future studies could follow children into adolescence to investigate if genetic influences start to emerge as children gain the independence to act in line with their genetically predisposed traits (a phenomenon termed 'gene-environment correlation') (Bergen, Gardner et al. 2007).

Given the importance of the shared environment for shaping EOE in early life, future research should seek to identify the actual influences at play. Previous studies have suggested that certain parental feeding practices are associated with higher EOE in children. Children whose parents actively control their child's emotions through feeding were found to engage more in EOE (Braden, Rhee et al. 2014; Tan and Holub 2015). Furthermore, parents who highly control the food intake of their children were found to elicit EOE behaviours (Farrow, Haycraft et al. 2015). Lastly, a stressful and chaotic home environment has been associated with childhood obesity, potentially because the child is provided with the type of environment in which a child would be more likely to learn to emotionally overeat (Gundersen, Mahatmya et al. 2011).

Limitations

All twin studies assume that MZ and DZ twins share their environments to the same extent (so-called the 'equal environments assumption') (Rijsdijk and Sham 2002). A recent analysis of past twin research testing for violation of the 'equal environments assumption' concluded that in the majority of studies reviewed no violations of the assumption occurred (Felson 2014).

The CEBQ is parent-reported and biases are therefore possible. For example, some of the shared environmental effect may reflect a parent's own tendency to emotionally overeat; on the other hand, parents may find it difficult to observe this behaviour with accuracy in young children, and therefore rate two twins the same. However, parents are well placed to report on their children's eating behaviour, arguably knowing their children better than other potential respondents. In addition, a range of other parent-reported eating behaviours showed high heritability in this sample during infancy (Llewellyn, van Jaarsveld et al. 2010); suggesting that parents are indeed able to observe differences between their twins for a range other eating behaviours, adding confidence to these

findings. Nevertheless, it would be useful to collect information from other raters in future studies (e.g. childcare providers), to compare with parental reports.

Another limitation of this study is the fairly low variation in EOE. Means of EOE at both time points were relatively low, suggesting a low occurrence of this behaviour in the sample. However the majority of children were found to engage in emotional overeating to some extent (16 months: 68.7% scored > 1; 5 years: 74.5% scored > 1). Low variation in a trait can impact the outcomes of twin modelling, as it potentially increases the similarity between twins regardless of zygosity, resulting in inflated shared environmental effects. However, previous research in Gemini assessing the heritability of eating behaviours in infancy, found that Enjoyment of Food was moderately heritable (53%), even though the behaviour showed low variation (being negatively skewed), with the majority scoring high (Llewellyn, van Jaarsveld et al. 2010). Here low variation was found not to result in an overestimation of shared environmental factors, and therefore supports the validity of the estimates in this study.

Additionally the high influence of shared environments supports the Psychosomatic Theory that hypothesizes that emotional overeating is largely learned in early life. If this is true, emotional overeating in early childhood may reflect emotional feeding by parents. Indeed, a positive relationship between emotional overeating and emotional feeding has been demonstrated in other samples (Braden, Rhee et al. 2014; Tan and Holub 2015).

A strength of this study is that in comparison to previous cross-sectional studies of adults, here participants were also the same age at the two assessment points. This is important as the relative contribution of different influences can vary widely during different developmental periods over the lifespan (Haworth, Carnell et al. 2008).

Conclusion

In summary this study suggests that variation in EOE during toddlerhood and early childhood is largely influenced by environmental factors shared by both twins in a family. In contrast to most other appetitive traits that have been explored in infancy and childhood, genetic effects play a minor role. Future studies are needed to identify the actual environmental factors influencing the development of EOE during the early years, and to elucidate when genetic influences start to emerge.

8 Planned work

8.1 Study 3. Genetic and environmental contributions to individual differences in EOE and EUE at five years

Study 3 in Chapter 8 was the first study to establish the relative importance of genes versus environments for EOE in childhood, at both 16 months and five years. I established that in early life, in contrast to adulthood, emotional overeating is largely driven by influences of the shared family environment. The relative influence of genes versus environments on EUE is currently unknown.

In addition, the nature of the relationship between EOE and EUE is unclear. These two behaviours tend to be positively correlated, but it is not known if they are different expressions of the same underlying trait (i.e. the tendency for one's appetite to be moderated by emotion, in terms of both up- and down-regulation), or they are distinct phenomena. As well as providing estimates of the relative importance of genes versus environments on variation in a single trait, twin studies can also be used to quantify the extent of shared genetic and environmental aetiology underlying different traits. Understanding the extent of shared aetiology underlying EOE and EUE would help researchers to understand the nature of the relationship between these two phenotypes.

8.1.1 Aims

The aims of this study are to: (i) estimate for the first time the genetic and environmental contributions to individual differences in EUE at five years, using a univariate twin model; (ii) to establish the phenotypic association between EOE and EUE at five years, and; (iii) to investigate the extent to which EOE and EUE share a common aetiology, using a bivariate twin model.

8.1.2 Method

EOE and EUE were measured at five years using the standard Child Eating Behaviour Questionnaire (CEBQ).

8.1.3 Analyses

The phenotypic association between EOE and EUE at five years will be investigated using Pearson's correlation coefficient.

8.1.3.1 Univariate twin model

Intraclass correlations will be calculated for MZ and DZ twin pairs for EUE at five years. Maximum Likelihood Structural Equation Modelling (MLSEM) will be used to derive reliable estimates of A, C and E with 95% confidence intervals, and provide goodness-of-fit statistics.

8.1.3.2 Bivariate twin model

To investigate the extent to which EOE and EUE share a common aetiology at five years, cross twin cross trait intraclass correlations will be calculated for MZ and DZ pairs (i.e. Twin 1's EOE will be correlated with Twin 2's EUE; and Twin 1's EUE will be correlated with Twin 2's EOE). MLSEM will be used to derive more reliable estimates of bivariate A, C and E, and aetiological correlations. The aetiological correlations will quantify the proportion of genetic (r_A), shared environmental (r_C), and unique environmental (r_E) factors that are the same for the two traits. The bivariate A, C and E estimates will quantify the proportion of the phenotypic correlation between EOE and EUE that is driven by common genetic (bivariate A), shared environmental (bivariate C) and unique environmental (bivariate E) factors underlying the two traits.

8.2 Study 4. Understanding the child, parental and wider family factors associated with emotional eating in childhood

Previous research has identified specific factors at different levels of influence that are associated with emotional overeating in childhood. As outlined in Chapter 2, child factors (including emotion regulation and food responsiveness), parental factors (such as parental feeding strategies), and family level factors (such as general stress in the home) have all been related to emotional over eating. The Gemini study has collected extensive data on various characteristics previously associated with emotional eating that can be combined to understand the interrelationships between these many factors. Additionally, the results of Study 2 suggest that variation in EOE is largely driven by environmental factors shared between twin pairs. I therefore hypothesise that parental factors (common to both twins) and wider family level factors, rather than individual child characteristics, will be of greater importance in determining EOE in childhood. Previous research on factors associated with EUE is limited and Study 3, which will establish the relative importance of genetic and environmental contributions to variation in EUE, is not yet completed. Therefore, at this stage it is not possible to hypothesise about the associations between EUE and child characteristics, parental and wider family factors.

8.2.1 Aims

The overall aim of this study is to examine together the interrelationships between child emotional over- and under-eating, and: (i) child characteristics (such as emotion regulation and food responsiveness); (ii) parental factors (such as their feeding styles, and their own tendency to emotionally overeat), and wider family level factors (such as stress in the home). I will explore both

cross-sectional and longitudinal associations between these characteristics and emotional over- and under-eating, to understand the direction of the relationship between these characteristics and emotional eating.

8.2.2 Methods

EOE behaviour was measured with the CEBQ-T at 16 months and the standard CEBQ at five years. EUE was measured with the standard CEBQ at five years. A multitude of different characteristics have been assessed in Gemini over the years, including child factors (strength and difficulties questionnaire, SDQ), parental factors (Parental Feeding Style Questionnaire, PFSQ; Dutch Eating Behaviour Questionnaire, DEBQ) as well as factors of the wider family environmental (Confusion, Hubbub and Order Scale, Chaos). Complex Samples General Linear Modelling will be used to explore the relationships between EOE, EUE and different child, parent and family level factors.

8.3 Study 5. Environmental risk and emotional eating: testing for genetic and environmental interplay in emotional overeating

The final study of my thesis will use the environmental risk factors (at the family level) identified for emotional overeating and for emotional undereating from Study 4 to create 'exposure' scores for both EOE and EUE. This will allow me to stratify the sample into children at high versus low risk of EOE and EUE, and to test if the genetic and environmental influences on EOE and EUE vary by environmental exposure, indicating gene-environment interaction in the shaping of emotional eating. Very few twin studies have explored gene-environment interaction, in the shaping of child behaviour.

8.3.1 Aims

The overall aim of this study is to test for gene-environment interactions in the expression of emotional over- and under-eating behaviour by testing if genetic and environmental influences on emotional over- and under-eating vary according to different environmental exposures.

8.3.2 Methods

Results from Study 4 will be used to create environmental risk scores for each of emotional overeating and emotional under-eating, for pairs of twins. Individual-level factors will therefore be excluded, and only family-level factors will be included. I will use these scores to divide twin pairs into those who experience high versus low exposure for EOE and EUE. I will use a heterogeneity twin model to test if genetic and environmental influences on EOE and EUE vary by environmental exposure.

8.4 Study 6. Understanding the interrelationships between emotional eating, food responsiveness and weight in early childhood

As discussed in detail in Chapter 2, the association between EOE and EUE and weight in childhood remains unclear. Multiple studies have investigated the relationship between emotional eating and weight, but findings are mixed (Wardle, Marsland et al. 1992; Braet and Van Strien 1997; Caccialanza, Nicholls et al. 2004; Braet, Claus et al. 2008; Jahnke and Warschburger 2008; van Strien and Oosterveld 2008; Viana, Sinde et al. 2008; Jollie-Trottier, Holm et al. 2009; Joyce and Zimmer-Gembeck 2009; Webber, Hill et al. 2009; Parkinson, Drewett et al. 2010; Sleddens, Kremers et al. 2010; Spence, Carson et al. 2011; Svensson, Lundborg et al. 2011; Cao, Svensson et al. 2012; Jansen, Roza et al. 2012; Snoek, Engels et al. 2013; Hajna, LeBlanc et al. 2014; dos Passos, Gigante et al. 2015; McCarthy, Chaoimh et al. 2015; Steinsbekk and Wichstrom 2015). Most previous research has been cross-sectional and more longitudinal studies are required to try to understand the nature of the relationships between emotional over- and under-eating, and adiposity. Furthermore, studies have shown that variation in the tendency to emotionally over- and/or under-eat emerges early, and is measurable. However, there are very few studies of the preschool period when these behaviours first start to develop.

The interrelationships between food responsiveness, emotional overeating and adiposity are also of interest. Food responsiveness (FR) (the desire to eat when food is in sight or presence of smell of food) in childhood has a well-established relationship with weight (French, Epstein et al. 2012), and prospective analyses in this sample have indicated that FR appears to play a causal role in weight gain in early life. Food responsiveness and EOE tend to be moderately positively correlated (e.g. r=0.49-0.54, Wardle, Guthrie et al. 2001; Sleddens, Kremers et al. 2008), and FR has a well-established positive relationship with childhood weight. Furthermore, EOE and FR have both been suggested to be influenced by the same parenting strategies (Jansen, Roza et al. 2012). It seems intuitive that children who are more responsive to food are more likely to be emotionally fed by their parents, and to learn to emotionally overeat. Emotional overeating may therefore be a behavioural phenotype that partly mediates the relationship between FR and EOE in children. This has not been explored.

8.4.1 Aims

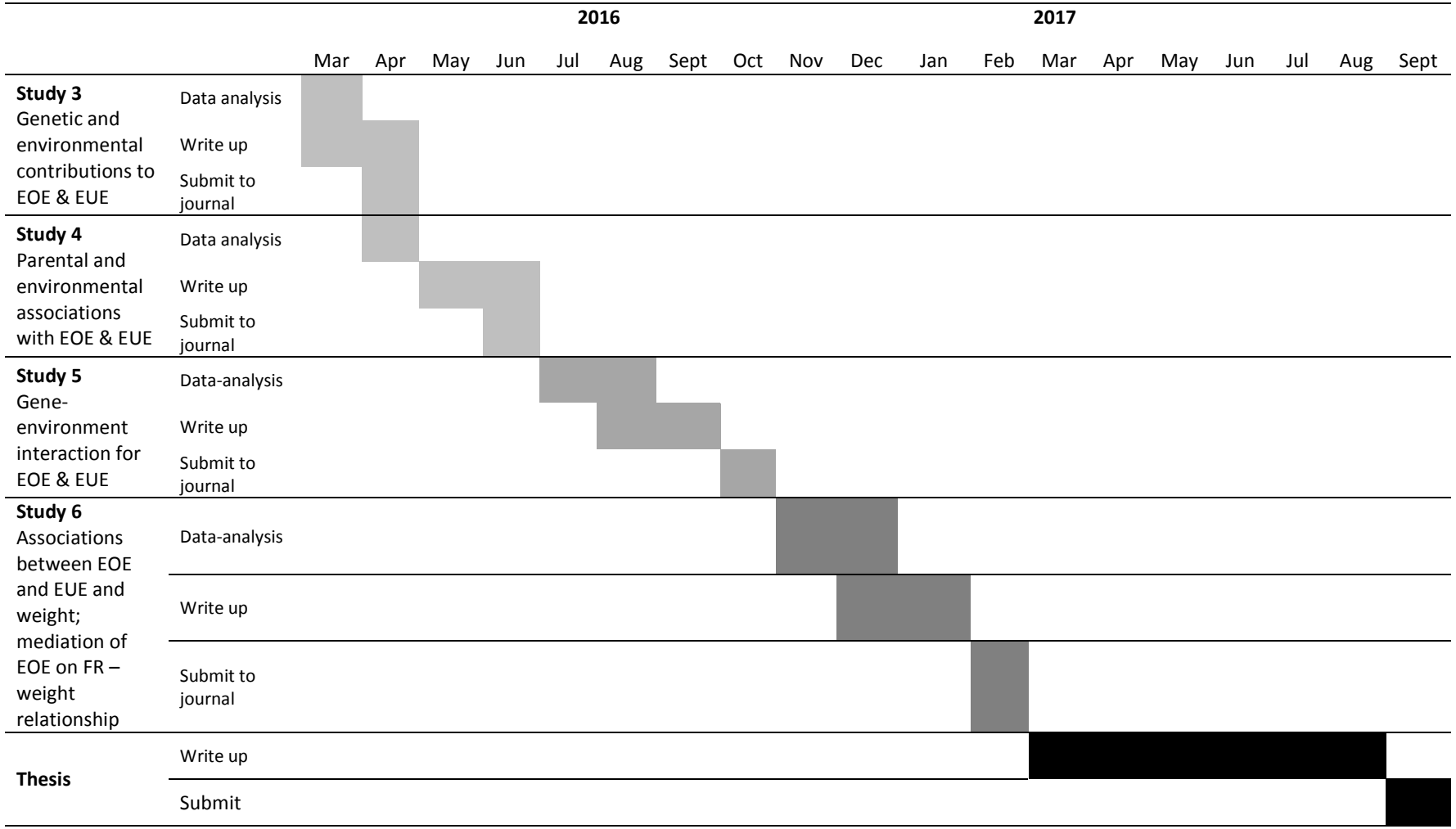
The overall aim of this study is to better understand the relationships between EOE, EUE and adiposity in toddlerhood and early childhood. There are four specific aims

a. To examine whether there is a cross-sectional association between emotional overeating and adiposity at 16 months.
b. To examine whether there are cross-sectional associations between emotional overeating and emotional under-eating and adiposity at five years.
c. To examine the longitudinal relationship between emotional overeating at 16 months with the development of adiposity from two to five years.
d. To test if EOE partly mediates the association between FR and weight in toddlerhood and early childhood, using cross-sectional (16 months and five years) and longitudinal analyses (from 16 months to five years).

8.4.2 Methods

EOE and FR were both measured with the CEBQ-T (16 months) and the CEBQ (five years). BMI-SDS at two and five years of age will be used to index adiposity. Complex Samples General Linear Models will be used to analyse the associations between EOE, EUE and adiposity, controlling for baseline covariates: age, sex, gestational weight, birthweight and maternal education. The Sobel test will be used to test if EOE significantly mediates the association between EOE and BMI-SDS.

9 Timeline for completion PhD



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11 Appendices

Appendix 1: Questionnaire items measuring EOE and EUE in childhood from the Family Activity and Eating Habit Questionnaire (FAEHQ), Child Eating Behaviour Questionnaire (CEBQ), Child Eating Behaviour Questionnaire-Toddler (CEBQ-T), Dutch Eating Behaviour Questionnaire – Children (DEBQ-C) and Dutch Eating Behaviour Questionnaire- Parent rated (DEBQ-P)

FAEHQ	CEBQ-T	CEBQ	DEBQ-C	DEBQ-P
How frequently does your child eat when angry or in other negative mood states	My child eats more when irritable	My child eats more when worried	Desire to eat when depressed	When your child is irritated, does he/she then have the desire to eat?*
How frequently does your child eat when bored	My child eats more when grumpy	My child eats more when annoyed	Desire to eat when feeling lonely	When your child has nothing to do, does he/she then have the desire to eat?*
	My child eats more when upset	My child eats more when anxious	Desire to eat when worrying	When your child is depressed or discouraged, does he/she then have the desire to eat?*
	My child eats more when s/he has nothing else to do	My child eats more when s/he has nothing else to do	Desire to eat when things go wrong	When your child is feeling lonely, does he/she then have the desire to eat?
		My child eats more when she is happy	Desire to eat when feeling restless	When your child feels let down, does he/she then have the desire to eat?*
		My child eats less when upset	Desire to eat when afraid	Has your child a desire to eat when he/she is cross?*
		My child eats less when angry	Desire to eat when feel sorry	When your child is expecting something unpleasant to happen does he/she then have the desire to eat?*
		My child eats less when s/he is tired		Does your child have the desire to eat when he/she is anxious, worried or tense?*
				When things are going against your child or when things have gone wrong, does he/she then have the desire to eat?

Appendix 1 (continued): Questionnaire items measuring EOE and EUE in childhood from the Family Activity and Eating Habit Questionnaire (FAEHQ), Child Eating Behaviour Questionnaire (CEBQ), Child Eating Behaviour Questionnaire-Toddler (CEBQ-T), Dutch Eating Behaviour Questionnaire – Children (DEBQ-C) and Dutch Eating Behaviour Questionnaire- Parent rated (DEBQ-P)

FAEHQ	CEBQ-T	CEBQ	DEBQ-C	DEBQ-P
				Does your child have the desire to eat, when he/she is emotionally upset'?*
				Does your child have the desire to eat when he/she is bored or restless?*
				When your child is frightened, does he/she then have the desire to eat?*
				When your child is disappointed, does he/she then have the desire to eat?*

Appendix 2 Questionnaire items measuring EOE in adulthood from the Dutch Eating Behaviour Questionnaire (DEBQ) and the Three Factor Eating Questionnaire (TEFQ)

DEBQ	TFEQ
Do you have the desire to eat when you are irritated	I start to eat when I feel anxious.
Do you have the desire to eat when you have nothing to do	When I feel sad, I often eat too much.
Do you have the desire to eat when you are feeling lonely	When I feel tense or "wound up", I often feel I need to eat.
Do you have the desire to eat when somebody lets you down	When I feel lonely, I console myself by eating.
Do you have the desire to eat when you are cross	If I feel nervous, I try to calm down by eating
Do you have the desire to eat when you are approaching something unpleasant to happen	When I feel depressed, I want to eat.
Do you have the desire to eat when you are anxious, worries or tense	
Do you have the desire to eat when you when things are going against you or when things have gone wrong	
Do you have the desire to eat when you are frightened	
Do you have the desire to eat when you are disappointed	
Do you have the desire to eat when you emotionally upset	
Do you have the desire to eat when you are bored or restless	

Appendix 3 The Child Eating Behaviour Questionnaire (CEBQ)

Child Eating Behaviour Questionnaire (CEBQ)

Please read the following statements and tick the boxes most appropriate to your child's eating behaviour.

	Never	Rarely	Some-times	Often	Always	
My child loves food	☐	☐	☐	☐	☐	EF
My child eats more when worried	☐	☐	☐	☐	☐	EOE
My child has a big appetite	☐	☐	☐	☐	☐	SR*
My child finishes his/her meal quickly	☐	☐	☐	☐	☐	SE*
My child is interested in food	☐	☐	☐	☐	☐	EF
My child is always asking for a drink	☐	☐	☐	☐	☐	DD
My child refuses new foods at first	☐	☐	☐	☐	☐	FF
My child eats slowly	☐	☐	☐	☐	☐	SE

My child eats less when angry	☐	☐	☐	☐	☐	EUE
My child enjoys tasting new foods	☐	☐	☐	☐	☐	FF*
My child eats less when s/he is tired	☐	☐	☐	☐	☐	EUE
My child is always asking for food	☐	☐	☐	☐	☐	FR
My child eats more when annoyed	☐	☐	☐	☐	☐	EOE
If allowed to, my child would eat too much	☐	☐	☐	☐	☐	FR
My child eats more when anxious	☐	☐	☐	☐	☐	EOE
My child enjoys a wide variety of foods	☐	☐	☐	☐	☐	FF*
My child leaves food on his/her plate at the end of a meal	☐	☐	☐	☐	☐	SR
My child takes more than 30 minutes to finish a meal	☐	☐	☐	☐	☐	SE

	Never	Rarely	Some -times	Often	Always	
Given the choice, my child would eat most of the time	☐	☐	☐	☐	☐	FR
My child looks forward to mealtimes	☐	☐	☐	☐	☐	EF
My child gets full before his/her meal is finished	☐	☐	☐	☐	☐	SR
My child enjoys eating	☐	☐	☐	☐	☐	EF
My child eats more when she is happy	☐	☐	☐	☐	☐	EUE
My child is difficult to please with meals	☐	☐	☐	☐	☐	FF
My child eats less when upset	☐	☐	☐	☐	☐	EUE
My child gets full up easily	☐	☐	☐	☐	☐	SR
My child eats more when s/he has nothing else to do	☐	☐	☐	☐	☐	EOE

Even if my child is full up s/he finds room to eat his/her favourite food	☐	☐	☐	☐	☐	FR
If given the chance, my child would drink continuously throughout the day	☐	☐	☐	☐	☐	DD
My child cannot eat a meal if s/he has had a snack just before	☐	☐	☐	☐	☐	SR
If given the chance, my child would always be having a drink	☐	☐	☐	☐	☐	DD
My child is interested in tasting food s/he hasn't tasted before	☐	☐	☐	☐	☐	FF*
My child decides that s/he doesn't like a food, even without tasting it	☐	☐	☐	☐	☐	FF
If given the chance, my child would always have food in his/her mouth	☐	☐	☐	☐	☐	FR
My child eats more and more slowly during the course of a meal	☐	☐	☐	☐	☐	SE

Food responsiveness (FR), Enjoyment of food (EF), Satiety responsiveness (SR) Slowness in eating (SE), Emotional Overeating (EOE), Emotional Undereating (EUE), Food Fussiness (FF), Desire to Drink (DD)

*Reversed items

Appendix 4 The Children's Eating Behaviour Questionnaire for Toddler (CEBQ-T)

CHILDREN'S EATING BEHAVIOUR QUESTIONNAIRE FOR TODDLERS (CEBQ-T)

How would you describe your child's eating styles on a typical day?

	Never	Rarely	Sometimes	Often	Always	
1. My child loves food	☐	☐	☐	☐	☐	EF
2. My child eats more when irritable	☐	☐	☐	☐	☐	EOE
3. My child has a big appetite*	☐	☐	☐	☐	☐	SR
4. My child finishes his/her meal quickly*	☐	☐	☐	☐	☐	SE
5. My child is interested in food	☐	☐	☐	☐	☐	EF
6. My child cannot eat a meal if he/she has had a snack just before	☐	☐	☐	☐	☐	SR
7. My child refuses new foods at first	☐	☐	☐	☐	☐	FF
8. My child eats slowly	☐	☐	☐	☐	☐	SE
9. My child looks forward to mealtimes	☐	☐	☐	☐	☐	EF
10. My child is always asking for food	☐	☐	☐	☐	☐	FR
11. My child eats more when grumpy	☐	☐	☐	☐	☐	EOE
12. If allowed to, my child would eat too much	☐	☐	☐	☐	☐	FR

13. My child eats more when upset	☐	☐	☐	☐	☐	EOE
14. My child enjoys a wide variety of foods*	☐	☐	☐	☐	☐	FF
15. My child leaves food on his/her plate or in the jar at the end of a meal	☐	☐	☐	☐	☐	SR
	Never	**Rarely**	**Sometimes**	**Often**	**Always**	
16. My child takes more than 30 minutes to finish a meal	☐	☐	☐	☐	☐	SE
17. Given the choice, my child would eat most of the time	☐	☐	☐	☐	☐	FR
18. My child enjoys tasting new foods*	☐	☐	☐	☐	☐	FF
19. My child gets full before his/her meal is finished	☐	☐	☐	☐	☐	SR
20. My child enjoys eating	☐	☐	☐	☐	☐	EF
21. My child is difficult to please with meals	☐	☐	☐	☐	☐	FF
22. My child decides that he/she does not like a food, even without tasting it	☐	☐	☐	☐	☐	FF
23. My child eats more and more slowly during the course of a meal	☐	☐	☐	☐	☐	SE
24. Even when my child has just eaten well, he/she is happy to eat again if offered	☐	☐	☐	☐	☐	FR
25. My child gets full up easily	☐	☐	☐	☐	☐	SR
26. My child is interested in tasting food he/she has not tasted before*	☐	☐	☐	☐	☐	FF

Food responsiveness (FR), Enjoyment of food (EF), Satiety responsiveness (SR), Slowness in eating (SE), Emotional Overeating (EOE), Food Fussiness (FF)

*Reversed items

Appendix 5 The Baby Eating Questionnaire

BABY EATING BEHAVIOUR QUESTIONNAIRE (BEBQ)

These questions are about your baby's appetite over his/her first few months of life. We are specifically interested in the period during which your baby is fed milk only, i.e. no solid foods or pre-prepared baby food yet.

How would you describe your baby's feeding style at a <u>typical daytime feed</u>?

	Never	Rarely	Sometimes	Often	Always	
1. My baby seems contented while feeding	☐	☐	☐	☐	☐	EF
2. My baby frequently wants more milk than I provide	☐	☐	☐	☐	☐	FR
3. My baby loves milk	☐	☐	☐	☐	☐	EF
4. My baby has a big appetite	☐	☐	☐	☐	☐	GA
5. My baby finishes feeding quickly*	☐	☐	☐	☐	☐	SE
6. My baby becomes distressed while feeding*	☐	☐	☐	☐	☐	EF
7. My baby gets full up easily	☐	☐	☐	☐	☐	SR
8. If allowed to, my baby would take too much milk	☐	☐	☐	☐	☐	FR
9. My baby takes more than 30 minutes to finish feeding	☐	☐	☐	☐	☐	SE
10. My baby gets full before taking all the milk I think he/she should have	☐	☐	☐	☐	☐	SR
11. My baby feeds slowly	☐	☐	☐	☐	☐	SE
12. Even when my baby has just eaten well he/she is happy to feed again if offered	☐	☐	☐	☐	☐	FR
13. My baby finds it difficult to manage a complete feed	☐	☐	☐	☐	☐	SR

14. My baby is always demanding a feed	☐	☐	☐	☐	☐	FR
15. My baby sucks more and more slowly during the course of a feed	☐	☐	☐	☐	☐	SE
16. If given the chance, my baby would always be feeding	☐	☐	☐	☐	☐	FR
17. My baby enjoys feeding time	☐	☐	☐	☐	☐	EF
18. My baby can easily take a feed within 30 minutes of the last one	☐	☐	☐	☐	☐	FR

Food responsiveness (FR), Enjoyment of food (EF), Satiety responsiveness (SR), Slowness in eating (SE), General appetite (GA)

*Reversed items