

**A LONGITUDINAL STUDY INVESTIGATING THE ROLE OF
BREASTFEEDING, POSITIVE MATERNAL INTERACTIONS
AND CORTISOL METABOLISM IN EATING BEHAVIOURS
AND WEIGHT GAIN IN INFANCY**

by

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A thesis submitted to the University of Birmingham for the degree of
DOCTOR OF PHILOSOPHY

School of Psychology
The University of Birmingham
September 2013

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For my Mom and Dad, Sue and Paul Rogers,
*because you have always had faith in me, sometimes more than I have had in
myself.*

ACKNOWLEDGEMENTS

First of all I would like to thank Dr Jackie Blissett. Jackie supported me in my pursuit of further education and has been an inspiration throughout. Jackie, you have frequently gone above and beyond what you could have as my supervisor. This thesis would not exist if it were not for your continuous support, encouragement and positivity. Thank you so much!

I would also like to thank Dr Jeremy Tomlinson for agreeing to collaborate with Jackie and me. Thank you Jeremy, for guiding us through the literature on steroid metabolism and for your continual support throughout this work. Thank you also to Bev Hughes for teaching me the process of steroid extraction – I shall never look at a nappy or potato ricer in quite the same way again!

I must also thank the mothers who took part in this study. I am extremely grateful to them all for inviting me into their homes and allowing me to witness their babies grow over their first year. This research could not have taken place without them. On a similar note, I must also thank the midwives at Birmingham Women's Hospital, particularly Paula Clarke, for their interest in the study and for facilitating recruitment.

I have the best family anyone could wish for. Mom and Dad, thank you for always being there – you have listened on stressful days and celebrated with me on successful ones. Thank you for your continued emotional, practical and financial support throughout my PhD. I *will* pay you back, somehow! My sisters Melanie and Abigail are fabulous characters and two of my very best friends. Thank you for spurring me on, and for never failing to make me laugh. Jav, your endless patience and belief in me renders me speechless. Thank you for being by my side and helping me to complete this. We can go on holiday soon, I promise!

Last but by no means least I would like to thank my friends, particularly Chloé, Gemma, Blaire, Julia, Charlotte and all of the girls in office 516. Thank you for your advice, encouragement and regular messages of support, you're all amazing!

ABSTRACT

Background: Previous research has shown that breastfeeding and positive maternal behaviours during feeding are associated with slower weight gain, healthy weight and the development of obesity-protective eating behaviours. Such research, however, has often not accounted for most significant covariates and many studies have suffered methodological problems, such as retrospective self-report of information. Furthermore, whilst cortisol (a bioactive ingredient of breast milk) has been related to eating behaviours and weight in human adults, there is no research investigating relationships between its metabolism and weight gain and eating behaviours during infancy.

Aims: The overall aim of this thesis was to investigate the roles of breastfeeding, positive maternal mealtime interactions and cortisol metabolism in weight gain and the development of eating behaviours during the first year of life, whilst controlling for covariates.

Methods: Eighty-one mothers and their new-born infants were recruited on low risk maternity units and seen/contacted at 1-week, 1-, 3-, 6- and 12-months postpartum. At every time point, feeding information was recorded and urine samples were collected for the analysis of cortisol metabolism. At the 1-week, 1-, 6- and 12-month home visits, mothers and infants were weighed and measured. Mothers were observed feeding their infants at 6- and 12-months and reported infant eating behaviours at 12-months.

Results: Mothers who breastfed for longer durations interacted more positively with their infants during a meal at 12-months; and had infants who were activating and

clearing more cortisol at 12-months and who gained weight more slowly throughout infancy. After accounting for breastfeeding duration, mothers who were observed to interact more positively had infants who were clearing, but not activating, more cortisol at 12-months. This suggests that maternal behaviour is involved in infant cortisol metabolism and therefore may have a programming effect on their infant's developing HPA axis. Regression analyses revealed that: slowness in eating was predicted by more cortisol clearance, via 5α -reductase; total observed food acceptances during a meal was predicted by less cortisol clearance, via 11β -HSD2; and slower infant weight gain was predicted by an increased duration of breastfeeding, lower level of maternal education, lower infant enjoyment of food and food fussiness and reduced clearance of cortisol via reduced 11β -HSD2.

Discussion: This thesis has provided the first set of normative data on the development of cortisol metabolism throughout the first year of life and has provided the first evidence that infant cortisol metabolism is an independent and significant predictor of eating behaviours and weight gain in the first year of life, even after accounting for key predictors such as breastfeeding duration and maternal interactions during feeding. Future research should investigate the long-term effects of (1) maternal behaviour on infant cortisol metabolism and (2) cortisol clearance on eating behaviours, weight and weight gain in infancy and early childhood.

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CHAPTER 1

LITERATURE REVIEW: WEIGHT GAIN, BREASTFEEDING, MATERNAL SENSITIVITY AND CORTISOL METABOLISM

The source of milk during infant feeding, maternal feeding practices, sensitivity of communication and quality of interactions all contribute to the development of eating behaviours and weight gain in childhood. Individuals with poor diets in childhood may be more at risk from serious health problems in adolescence and adulthood (World Health Organisation; WHO, 2003a). Given that eating behaviours have been shown to persist from childhood to adulthood (Ashcroft, Semmler, Carnell, van Jaarsveld & Wardle, 2008; Lien, Lytle, & Klepp, 2001) and that obese children are more likely than their non-obese counterparts to become obese adults (Freedman, Dietz, Srinivasan & Berenson, 1999), it is of great importance to further understand the contributors to obesogenic eating behaviours and excessive weight in infancy.

This literature review will begin by describing how growth is measured in infancy, the prevalence and consequences of overweight and obesity and how breastfeeding may help to prevent these conditions later in life. The literature review will then discuss the contribution of maternal behaviours to infant weight gain and eating behaviours before then introducing the argument that the *metabolism* of cortisol, a hormone present in breast milk, may have an effect on weight gain and eating behaviours during infancy. This chapter will then detail the demographic and psychological variables that are essential to consider when investigating weight gain and feeding

behaviours before summarising the gaps in current research and stating the aims and hypotheses of the thesis.

1.1 Infant growth

Before discussing the prevalence and consequences of childhood obesity, it is first of all important to understand the more general predictors of weight gain in infancy and early childhood, and how growth is measured in infancy and the preschool years. The amount of weight an infant gains in the early postnatal period and the speed of this weight gain is influenced by a variety of pre- and postnatal factors. Examples of these factors include: maternal nutritional and smoking status during pregnancy (Oken, Levitan & Gillman, 2008; Yang & Huffman, 2013); infant's own health and birth weight (Rogers, 2003); protein content of the breast- and/or formula-milk the infant is being fed (Koletzko et al., 2011); parental, particularly maternal, BMI and adiposity (O'Reilly & Reynolds, 2013); maternal psychopathology (Wright, Parkinson & Drewett, 2006); and infant's sleep duration (Taveras, Rifas-Shiman, Oken, Gunderson & Gillman, 2008).

The UK-WHO growth charts have been used by health professionals in the UK since May 2009 (Appendix 1). Whereas previous growth charts had been based on data from studies of breast- and formula-fed children, the new charts are now based on the WHO Child Growth Standards and describe optimal growth for healthy breast-fed children. The new charts combine UK90 and WHO data and better reflect the normal weight fluctuations, seen in the first few weeks of life of breast-fed infants (Royal

College of Paediatrics and Child Health; RCPCH, 2009). As can be observed from these charts, girls at 12-months-old will be in or above the 98th centile for weight if they weigh 11.5kg or more; 12-month-old boys will be in or above the 98th centile for weight if they weigh 11.9kg or more (Appendix 1).

The WHO produced new charts because breastfed infants appeared to be growing less optimally when plotted on charts based on data from both breast- and formula-fed infants. It was also observed that healthy breastfed infants demonstrated similar growth patterns all over the world (RCPCH, 2009). It was therefore decided to create charts that set breastfeeding as the norm and could be used internationally (RCPCH, 2009). The new charts were created based on data from healthy infants, born at term, to non-smoking mothers living in comfortable economic circumstances. Infants were required to have been exclusively breastfed for a minimum of 4-months, followed by partial breastfeeding for 1-year, and were introduced to solid food by 6-months-old. Infants were from the USA, Norway, India, Ghana, Brazil and Oman (RCPCH, 2009). The range of countries involved in the creation of the growth charts is likely to be advantageous when using them in the UK, given its diverse population. Once created, the charts were studied to see how children in the UK compared to them. It was decided that the new charts would be used from 2-weeks postpartum so that the UK 1990 preterm and term birth data could still be used. The UK 1990 charts would be used again from 4-years of age so that school entry measures could continue to be plotted (RCPCH, 2009). The new charts were designed by the RCPCH, which span from 32-weeks gestation to 4-years-of-age (RCPCH, 2009).

From the new charts, it can be observed that breastfed infants grow more slowly, rather than less optimally, than formula-fed infants. Breastfed infants show little rapid immediate postnatal growth, in fact they often lose weight in the immediate postnatal period. For example, MacDonald, Ross, Grant and Young (2003) examined neonatal weight loss in breast and formula-fed infants. Infants were weighed by midwives at birth and at 2-, 5-, 7- and 10-days postpartum; weighing of infants ceased once they had returned to their birth weight. The authors found that breastfed infants lost more weight in the early postnatal period than formula-fed infants. It was also found that formula-fed infants were quicker to regain the weight that had been lost compared to breastfed infants. Given the differences between breast and formula fed infants in their pattern of growth, it is perhaps unsurprising that breastfeeding has a protective effect against obesity and obesogenic eating behaviours in later life (Arenz, Ruckerl, Koletzko, & von Kries, 2004; Owen, Martin, Whincup, Davey Smith & Cook, 2005). Literature on early overweight and obesity, and breastfeeding's role in obesity, is reviewed next.

1.2 Overweight and obesity

Obesity is a global epidemic (WHO, 2000) and its prevalence has almost doubled worldwide since 1980 (WHO, 2013). Overweight and obesity are not only problems of high-income countries; rates are now increasing in middle- and low-income countries also (WHO, 2013). In the United Kingdom (UK), the prevalence of obesity in children aged between 2- and 10-years-of-age rose from 9.9% in 1995 to 14.3% in 2004 (Department of Health, 2006); in 2010, 30% of 2- to 15-year-olds were classed as

overweight or obese (Heaton, 2013). More recent data indicates that the prevalence of child obesity in England has been slowing in its rate of increase since 2004 (Public Health England, 2013).

As infectious and nutrient diseases are being more adequately treated, health problems related to obesity are on the rise (WHO, 2000). It is known that as body mass index (BMI) increases (especially above 30kg/m²), mortality rates increase; higher BMI is associated with an elevated risk of type 2 diabetes, higher blood pressure and risk of hypertension, higher total cholesterol, higher low-density lipoprotein cholesterol and triglyceride levels and lower high-density lipoprotein cholesterol levels (Jung, 1997). Obese individuals are therefore at a greater risk of health problems including: coronary heart disease, stroke, gout, sleep apnoea and obstetric and surgical complications (Jung, 1997). Furthermore, these problems will have a large impact both directly and indirectly on health care costs. Overweight children and adolescents already demonstrate elevated blood pressure, cholesterol, triglyceride and insulin levels (Freedman et al., 1999).

In addition to this, childhood obesity is a predictor for adult obesity (Freedman et al, 1999); 80% of children who are obese between 10- and 14-years-of-age become obese adults, particularly if one of their parents is also obese (Whitaker, Wright, Pepe, Seidel & Dietz, 1997). Early infant weight gain appears to be a critical determinant of later weight, and infants who grow more rapidly are also at risk of a variety of later diseases (Cole, 2007). Although obesity prevalence in children appears to be reaching a plateau (Public Health England, 2013), because it is not

significantly reducing and rates of overweight remain unchanged, it is important to understand its aetiology to develop effective preventions and interventions. It is of great importance that any factors linked to a reduced risk of paediatric obesity and obesogenic eating behaviours are further investigated. Obesogenic eating behaviours may include decreased self-regulation of intake, decreased fruit and vegetable consumption, disinhibition and eating in the absence of hunger.

1.2.1 The first year of life

The present study will focus on the first year of life because it is the first few weeks to months of life that is believed to be important in developing obesity later on. It has been found that rapid weight gain during the first weeks or months of infancy predicts obesity later in life (Cole, 2007; Lanigan & Singhal, 2009; Taveras et al., 2009). Ay et al., (2009) measured body composition and weight of 252 infants who were involved in a larger prospective cohort study. It was found that catch-up growth within 6-weeks from birth was associated with an increase in fat mass at the age of 6-months. In addition to this, Gillman (2008) reviewed studies and concluded that the first few weeks to months of life are a sensitive period for the development of obesity. Two of the studies reviewed include: Botton et al., (2008) who found weight gain velocity at 3- and 6-months predicted adolescent fat mass better than weight gain velocity at 1- or 2-years; and Bhargava et al., (2004) who discovered BMI increase in the first 6-months was related to BMI and sum of skinfold thicknesses in adulthood.

Hales and Barker (1992 as cited in Hales & Barker, 2001) proposed the thrifty phenotype hypothesis (Figure 1.1). The thrifty phenotype hypothesis explains that adverse conditions during prenatal and early postnatal development programme the infant's physiology, increasing its risk of developing diseases such as obesity, type 2 diabetes and cardiovascular diseases later on in life. Plagemann (2004; 2012) terms this 'perinatal programming'. As illustrated in Figure 1.1, Hales and Barker (1992 as cited in Hales & Barker 2001) describe that inadequate foetal and infant nutrition, caused by maternal malnutrition and other maternal/placental influences, programme the infant to expect and cope with an environment characterised by poor nutrition. This early life malnutrition is suggested to hamper the development of pancreatic β -cell quantity and function; reduced β -cell function is one of the crucial factors linking early malnutrition to later type 2 diabetes.

It is also suggested that foetal malnutrition leads to insulin resistance. Impaired insulin secretion and sensitivity would not be damaging to individuals who continued to be poorly nourished and remained thin; adaptations become harmful however, when the postnatal environment differs from the one in which the infant has been programmed for. If, as in Western societies for example, individuals are later confronted with an environment that is over-abundant with nutrients, this may lead to obesity, type 2 diabetes and metabolic syndrome (Hales & Barker 1992 as cited in Hales & Barker 2001). Whilst this effect is seen more strongly in low birth weight infants, it must be noted here that these effects do occur across a range of birth weights including those born within the normal weight range (Hales & Barker, 2001).

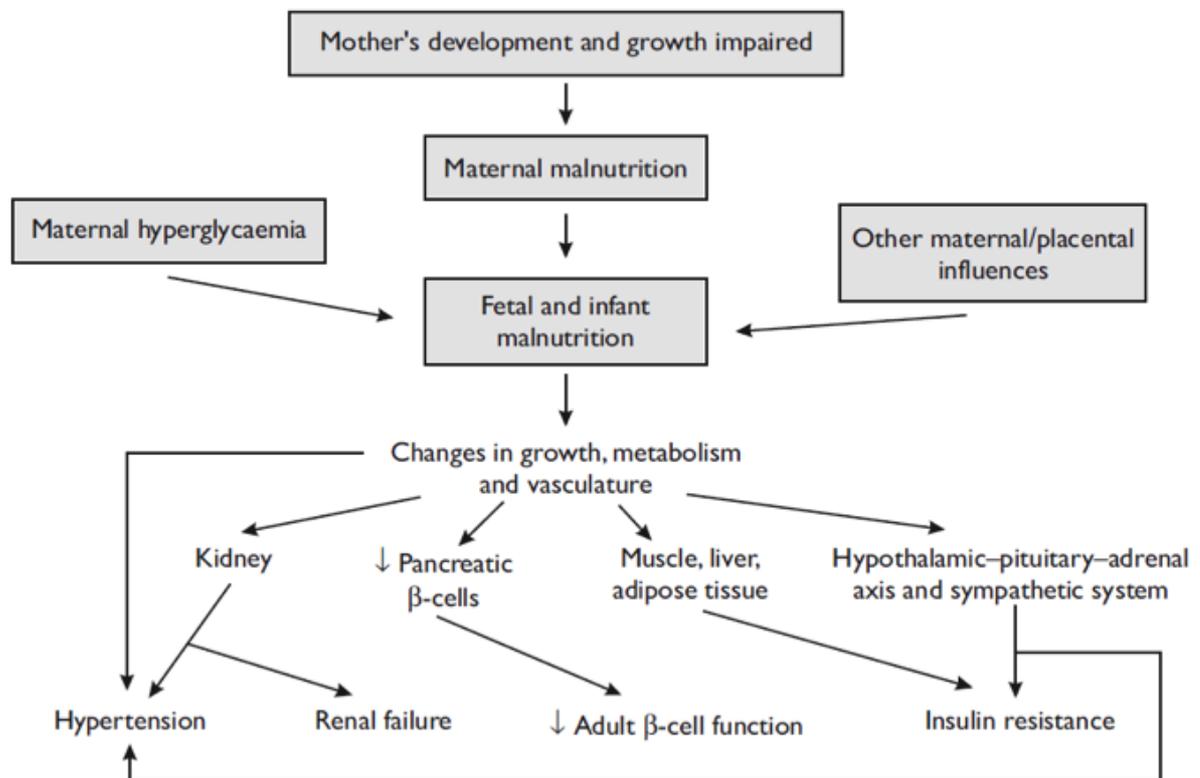


Figure 1.1

Updated diagram of the thrifty phenotype hypothesis as pictured in Hales and Barker (2001)

In addition to their internal characteristics, environmental factors, such as parental behaviours, also influence children's weight gain. Rothbaum and Weisz (1994) stated parents' caregiving and children's characteristics are continually exerting a pull on one another and, over time, these behaviours become increasingly interwoven. The older the child, the more difficult it is to disentangle the relationship between child weight, eating behaviours and other influential factors. It has also been found that childhood obesity has a larger impact on the development of metabolic syndrome than does obesity in adulthood (Vanhala, Vanhala, Kumpusalo, Halonen & Takala, 1998). As previously mentioned, the rate of weight gain in the first weeks to months

of life has been shown to be related to later childhood overweight (Dennison, Edmunds, Stratton & Pruzek, 2006; Stettler et al., 2005). However, the causes of different patterns of weight gain in early infancy are not entirely understood.

1.2.2 Breastfeeding

In addition to the fact that breastfeeding has a protective effect against obesity and obesogenic eating behaviours in later life (Arenz et al., 2004; Owen et al., 2005), breastfeeding also reduces the risk of infants developing eczema, ear and chest infections and stomach bugs; mothers who breastfeed have a reduced risk of breast and ovarian cancer and can find it easier to lose weight due to breastfeeding using up to 500 extra calories per day (Start 4 Life, 2012). Due to these well-documented benefits, the government and health service in the UK are increasing the promotion of breastfeeding.

The Infant Feeding Survey is conducted in the UK every 5-years and has been since 1975. The main aim of this survey is to estimate information on the incidence, prevalence and duration of breastfeeding (and other feeding methods) during the first 8- to 10-months of an infant's life. The Infant Feeding Survey 2010 was based on a representative sample of mothers who were selected from all registered births in the UK between August and October 2010 (McAndrew et al, 2010a). Compared to the data collected from the previous survey in 2005, it was observed in 2010 that: the proportion of breastfeeding at birth in the UK rose from 76% to 81%; exclusive breastfeeding rose from 13% to 17% at 3-months and from 7% to 12% at 4-months;

any breastfeeding rose from 48% to 55% at 6-weeks and from 25% to 34% at 6-months. Breastfeeding was observed to be more common in mothers who: were aged over 30-years; were from minority ethnic groups; had remained in education beyond the age of 18; were in managerial or professional occupations; and who lived in areas that were not deprived (McAndrew et al, 2010a).

Research has shown that women in the UK cease breastfeeding sooner when: they work as employees (rather than those who are self-employed or do not work at all [Skafida, 2012]); they have lower confidence in their ability to successfully breastfeed; those around them more often bottle-feed rather than breastfeed; they do not have professional support (Entwistle, Kendall & Mead, 2010); they are primiparous rather than multiparous (Agboado, Michel, Jackson & Verma, 2010); and when they had decided prenatally that they did not intend to breastfeed (Donath & Amir, 2003). Other reasons why women do not continue with, or even start, breastfeeding include inadequate training of staff who work within the health service in the support of breastfeeding women (Renfrew et al., 2006) and also the representation of formula-feeding as being the normal feeding practice by the media (Henderson, Kitzinger & Green, 2000). Henderson et al., (2000) looked at 235 references to infant feeding on television programmes in the UK in March 1999. It was found that bottle-feeding was shown more often than breastfeeding and that bottle-feeding was portrayed as less problematic.

The first study documenting breastfeeding's protective effect against obesity was published by Kramer (1981), who found that those who were not breastfed were at a

higher risk of becoming obese and this protective effect of breastfeeding increased slightly as the duration of breastfeeding increased. Since then, a large amount of literature has been published investigating this relationship. A meta-analysis of seventeen studies conducted by Harder, Bergmann, Kallischnigg and Plagemann (2005) strongly supported the conclusion that a longer duration of breastfeeding is associated with a decreased risk of overweight. It was found that for each month of breastfeeding, the risk of becoming overweight was reduced by 4% (Harder et al., 2005). Furthermore, a study of 420 children aged from 6-months to 10-years by Novotny et al., (2007) found children who had been breastfed had lower BMIs than those who had not been breastfed. Analysis of cross sectional data has also demonstrated that breastfeeding is protective against overweight and obesity (Twells & Newhook, 2010; Simon, de Souza & Souza, 2008). Simon et al., (2008) found this effect in their sample of 566 2- to 6-year-old children in Brazil and Twells and Newhook (2010) demonstrated this in 1,026 4-year-olds in Canada.

Data from the DARLING Study has revealed that breastfed infants demonstrate lower weight gain during the critical neonatal period, due to a lower mean caloric intake compared to infants who are fed formula milk (Heinig, Nommsen, Peerson, Lönnerdal, & Dewey, 1993). The DARLING Study was designed to longitudinally assess growth, nutrient intake, activity and morbidity of infants who received breast- and/or formula-milk beyond the age of 12-months. Data from this study highlighted that breastfed infants were found to exhibit significantly lower weight gain between 3- to 6- and 6- to 9-months, than formula-fed infants (Heinig et al., 1993). Lower weight gain during this time has been linked to a reduced risk of obesity later in life (Stettler,

Zemel, Kumanyika, & Stallings, 2002). In support of these findings, Hörnell, Lagström, Lande and Thorsdottir (2013) conducted a systematic literature review and found there to be convincing evidence of a dose-dependent protective effect of breastfeeding on overweight and obesity in later childhood and adolescence. The authors stated this reduced risk may be partly explained by the evidence that exclusive breastfeeding for at least 4-months is associated with slower weight gain in the latter half of the first 12-months of life.

The mechanisms by which breastfeeding confers this small protective effect may include: the caloric and protein content of the milk, the infant learning to attend to internal signals of hunger and satiety, facilitation of maternal sensitivity, maternal experience of allowing the infant to control its own intake, and/or the biologically active ingredients within breast-milk. Formula milk has been found to contain double the amount of protein of breast milk and protein intake is 50-80% higher in formula-fed versus breast-fed infants (Alexy, Kersting, Sichert-Hellert, Manz & Schoch, 1999).

The EU Childhood Obesity Programme (EUCOP; 2007) found that infants consuming higher quantities of protein in formula milk had greater weight-for-length and BMI at 12- and 24-months than those receiving a third less through either formula milk containing lower protein or breast milk. It is important to note that there were no differences in total energy or carbohydrate content of low protein formula, high protein formula or breast milk (EUCOP, 2007). Whitehead (1995) also found that breastfed infants demonstrate lower protein intake and energy metabolism than formula-fed infants. Given these observed differences, it is possible that the

difference in ingredients of breast versus formula milk combined with behavioural variables, such as increased infant control and maternal sensitivity, during feeding may influence the development of healthier eating styles and patterns of weight gain.

Breastfeeding enables the infant to learn and react to hunger and satiety cues and apply more control over initiation and termination of feeding (Birch & Fisher, 1998). When infants are formula-fed, parents have greater control over the amount of milk consumed, including the concentration of the formula and encouraging them to finish the whole bottle. Therefore, children that are breastfed may learn to self-regulate caloric intake better than children that are fed formula milk (Birch & Fisher, 1998). In addition to this, for infants who are fed formula milk, they consume meals that are similar, if not the same, in volume and energy content; breastfed infants on the other hand do not, due to the fact that breast milk changes in energy content throughout the day and within each feed (Jenness, 1979; Nommsen, Lovelady, Heinig, Lönnerdal & Dewey, 1991). Breastfed infants therefore learn to adapt their intake and have been found to consume less milk if it is higher in fat content (Tyson, Burchfield & Sentence, 1992).

In support of this, Brown and Lee (2012) investigated relationships between infant milk feeding and maternal report of infant satiety and food responsiveness. Between 6- and 12-months postpartum, mothers reported breastfeeding duration and exclusivity and weaning information; when infants were aged between 18- and 24-months, mothers reported infant satiety and food responsiveness via questionnaire. From the 298 mothers included in the analysis, it was found that infants breastfed for

longer durations were rated as being more satiety responsive. It was also found that a minimum duration of 6-weeks of breastfeeding was required for increased satiety responsiveness to be observed. However, a significant association between infant current weight and breastfeeding duration was not found. It is important to consider that Brown and Lee (2012) did not control for the age at which infants were introduced to solid food in their analyses. Earlier introduction of solid food has been related to greater weight gain during the first year of life (Baird et al., 2008; Baker, Michaelsen, Rasmussen & Sorensen 2004; Forsyth, Ogston, Clark, Florey & Howie 1993; Kramer et al., 1985; Lande et al., 2005); solid food introduction will be discussed in more detail in the next section of this literature review.

The evidence for the protective effect of breastfeeding on long term obesity and weight gain however, is not entirely conclusive. Oddy et al., (2004) looked at follow-up data from 2,195 6-year-old children involved in a prospective birth cohort study in Western Australia; the authors found no association between breastfeeding and later overweight. Davis et al., (2007) investigated relationships between breastfeeding and diabetes risk in 240 overweight Latino children who were aged between 8- and 13-years-of-age. The authors did not find any significant effects of breastfeeding on adiposity. However, 74% of the sample was male, and although male and female Latino children both have high rates of obesity, it has been observed that Latino boys are at an increased risk of becoming overweight when compared to boys from other ethnic backgrounds (Aguirre-Molina & Betancourt, 2010).

Martin et al., (2013) found that an intervention successful in improving duration and exclusivity of breastfeeding did not prevent overweight or obesity when children were 11.5-years-old. It is however, important to consider that the equivocality in the literature may be due to a range of methodological problems and issues relating to the control of extraneous and confounding variables within studies. For example, Oddy et al., (2004) did not control for maternal weight or BMI and Davis et al., (2007) did not control for maternal weight, BMI, smoking during pregnancy or socioeconomic status. There is a wide variation in the extent to which potential confounding variables have been adjusted for across studies. Examples of such variables in this literature include: child/infant gender, ethnicity, birth weight and gestational age, maternal age, BMI, smoking status during pregnancy (and postnatally), maternal diabetes, educational level and household income. Examples of these factors will be discussed in more detail later in this literature review.

In addition to the extraneous and confounding variables within studies, there are also other methodological issues within this area of research. Examples include: how obesity is defined, different BMI cut-off points and percentiles used, retrospective data collection, inconsistent definitions of breastfeeding between studies (including exclusivity and duration), small sample sizes or the same data from larger samples being used in a number of studies, and self-report of height, weight and breastfeeding history. Another frequent methodological problem throughout the literature is how breastfeeding is defined. Many samples used are taken from large (prospective) birth cohort studies, the aims of which are not specifically to investigate the potential protective effect of breastfeeding on obesity. Subsequently this means

that breastfeeding is often not accurately defined in terms of exclusivity and duration even within individual studies.

In many studies, much of the data regarding breastfeeding has been collected through retrospective self-report measures using samples which have been studied several times. For example, Michels et al., (2007) sent out questionnaires to nurses' mothers asking if they breastfed their daughters and when breastfeeding stopped. It is important to draw attention to the fact that nurses' mothers were contacted when the nurses were aged between 37- and 44-years. The accuracy of such retrospective self-report is therefore questionable. Cohorts that have been used more than once in the examination of the relationship between breastfeeding and weight gain include: ALSPAC (Ong, Emmett, Noble, Ness & Dunger, 2006; Toschke et al., 2007); NLSY79 (Li, Goran, Kaur, Nollen & Ahluwalia, 2007; Li et al., 2005); Project Viva (Li et al., 2007; Taveras et al., 2006); and the Growing Up Today Study (Gillman et al., 2001; Gillman et al., 2006; Mayer-Davis et al., 2006). Furthermore, the participants of the Growing Up Today Study are children of participants of the Nurses' Health Study II, a study that has also been used in this research area (Michels et al., 2007). This means that not only are some samples being used multiple times to test the same variables, but the same environmental and genetic information is contributing to two separate samples, both of which have been studied numerous times. The use of the same cohort more than once partially explains the occurrence of repeated findings both for and against the protective effect of breastfeeding on obesity; if one sample has generated inconclusive or negative results for example, then it would not be

surprising that if employed again, the same sample would again have a similar outcome.

However, whilst bearing in mind the methodological shortcomings of the current literature, it still appears that there is a small protective effect of breastfeeding on overweight and obesity. This is highlighted by the results of a meta-analysis by Harder et al., (2005) who found a dose-dependent relationship between a longer duration of breastfeeding and a decreased risk of becoming overweight and a systematic review by Owen et al (2005) who concluded that initial breastfeeding does have a protective effect on obesity later in life. Therefore the mechanisms by which breastfeeding are proposed to protect against obesity and obesogenic eating behaviours require further research in order to aid the prevention of paediatric obesity.

Breastfeeding is not only associated with weight status later in life, but the development of adaptive eating behaviours. Kudlová and Schneidrová (2012) for example, found that breastfeeding is associated with higher fruit and vegetable intake frequency in children. This relationship is likely to have arisen because breast milk consists of flavours that are directly related to the foods and drink consumed by the mother. Breastfeeding may therefore aid the consumption of healthier diets by influencing the development of food preferences through milk-related flavour exposures (Nicklaus, 2009). Mennella, Jagnow and Beauchamp (2001) found that, when fed a carrot-flavoured cereal, infants who had been exposed to the flavour of carrots in breast milk demonstrated fewer negative facial expressions than infants

who had not been exposed. Breastfeeding may therefore confer a more general protective effect over healthful eating behaviours. To progress this research further, it is necessary to investigate behavioural and biological factors related to breastfeeding to establish if, and how, they are mechanisms for this protective effect.

1.2.3 Introduction to solid food

As previously mentioned, introducing solid food before the infant is 4-months-old can increase their risk of later obesity (Ong et al., 2006; Huh, Rifas-Shiman, Taveras, Oken, & Gillman, 2011). Brown and Lee (2012) propose that earlier introduction of solid food may encourage infants to consume a larger amount of energy, which may then interfere with the infant's self-regulation of energy intake. Interestingly, Huh et al., (2011) found that introducing solids before 4-months is only associated with an increased risk of childhood obesity in formula-fed, and not breastfed, infants. Such results suggest that not only are formula-fed infants likely to be heavier than their breastfed counterparts as they get older, but earlier introduction to solid food will further increase their risk of becoming overweight in childhood. Therefore, it is plausible that longer durations of breastfeeding may promote increased satiety responsiveness, which combined with introducing solid food at the optimal time, may promote increased self-regulation of intake and healthier patterns of weight gain.

It is important to consider here however, that it can be difficult to draw specific conclusions from research in this area, unless studies allocate different weaning groups. Mothers respond to their infant's cues, therefore those that introduce solid

food early may do so because they have a hungrier and more demanding baby. These hungrier infants are likely to be less satiety responsive, even before they are weaned.

Although research has shown that introducing infants to solids 'early' enhances later obesity risk, the optimal timing of solid food introduction has been debated. Advice tends to differ between countries and cultures (Schwartz, Scholtens, Lalanne, Weenen & Nicklaus, 2011). Recommendations have included: not before 17-weeks but no later than 26-weeks (Agostoni et al., 2008); and after the infant is 6-months-old and never before 4-months-old (WHO, 2001; 2003b). In the UK, it is currently recommended to introduce solid foods when infants are around 6-months-old.

The 2010 Infant Feeding Survey showed that UK mothers were introducing their infants to solid food later than they were in 2005. In 2005, 51% of 4-month-olds had been introduced to solids, whereas this fell to 30% in 2010 (McAndrew et al., 2010b). Early weaning was found to be more prevalent in mothers who were younger and of lower socioeconomic status. Key reasons reported for introducing their infants to solids included: infants were no longer satisfied with milk alone; mothers had had previous experience with another baby; the infant was able to sit upright and hold his/her own food; advice from a health professional; and the infant had been waking during the night. The majority of mothers surveyed stated that the first food they fed their infant was either mashed or pureed; whereas only 4% gave finger food (McAndrew et al., 2010b).

Despite various recommendations, the move to feeding solid foods is recognised as a critical period in preparing the infant for a varied diet in order to optimise health and growth. A detailed report on the guidelines and practice of solid food feeding was produced by Hetherington, Cecil, Jackson and Schwartz (2011). As this report highlights, research has demonstrated that there are ideal periods to introduce specific tastes and textures to facilitate the development of healthy eating behaviours. Harris (1993) for example, proposed that there are certain times whereby infants more readily accept particular tastes and textures. Furthermore, the timing and introduction of foods (reported at 6-months postpartum) has been found to affect dietary choice and fussiness when children are 7-years-old (Coulthard, Harris & Emmett, 2009, 2010).

In addition to the timing of introducing solid foods, the types of food being introduced are also important (Hetherington et al., 2011). Coulthard et al., (2010) found that exposing infants to home-prepared fruits and vegetables, as opposed to those ready-prepared, in the early weaning period (about 6-months) results in higher proportion of fruit and vegetable consumption at 7-years-old. Furthermore, Sullivan and Birch (1994) found that infants increase their consumption of a novel vegetable after repeated exposures to it over a 10-day period. Interestingly, breastfed infants demonstrated greater increases in intake of the vegetable than formula-fed infants. The authors concluded that breastfeeding may facilitate acceptance of novel foods and suggested that this observable difference may be the result of milk-related flavour exposure. Research therefore demonstrates that breastfeeding for longer durations and exposing infants to a range of solid foods, at the optimal time,

enhances the likelihood of the infant developing healthy eating behaviours and patterns of weight gain. A mechanism to consider in this positive relationship is maternal sensitivity and control during feeding.

1.2.4 Maternal sensitivity and control

1.2.4.1 Maternal sensitivity to infant cues

A further factor involved in the development of overweight and obesity is maternal sensitivity and control over infant feeding. Maternal sensitivity refers to how accurately mothers perceive and interpret the signals and communications of their infant, and if these signals and communications are responded to in a prompt and appropriate manner (Ainsworth, Bell & Stayton, 1979). Maternal sensitivity is important during feeding because insensitive mothers may either not pick-up on, or may ignore, infant signals, which could then override the infant's internal hunger and satiety cues. Taking this into account, maternal sensitivity may be one of the mechanisms by which breastfeeding protects against obesity and obesogenic eating behaviours.

It is possible that mothers who formula-feed may be less sensitive, demonstrate greater control over feeding and be more likely to overfeed their infants. Overfeeding is more likely to occur when infants are formula-fed, as these mothers may be more likely to rely on visual cues regarding the amount of milk left in the bottle and ignore the satiety signals of their infant (Dewey, 2001). In support of this, in a sample of

formula-feeding mothers, regular overfeeding of infants was indicated by maternal reports of feeding frequency and reduced sensitivity to infant cues (Worobey, Lopez & Hoffman, 2009). Infants who become overweight have been found to consume 42% more energy at 6-months than those who remain lean (Roberts, 1991).

On the other hand, mothers who breastfeed their infants may be more responsive to their infants' signals regarding feeding. Breastfeeding mothers demonstrate less controlling feeding practices, allowing their infants to better self-regulate their energy intake and learn to respond to internal cues of hunger and satiety (Brown & Lee, 2013; Taveras et al., 2006). DiSantis, Hodges and Fisher (2013) investigated links between breastfeeding and feeding styles in 154 mothers of infants and toddlers. Mothers self-reported breastfeeding history and feeding approaches via questionnaire. The authors found that longer durations of breastfeeding were related to greater reported maternal responsiveness to infant satiety cues.

In a large sample of 1,160 mother-infant dyads, Taveras et al., (2004) found the longer mothers breastfed, the less likely they were to restrict their children's food intake at 1-year-of-age, a practice which has been linked to later weight gain and poor self-regulation of food intake (Birch & Fisher, 1998). In support of this, breastfed infants have been shown to be able to better adjust their food intake to a high caloric preload (Birch & Fisher, 1998). Farrow and Blissett (2006a) also found that breastfeeding, mediated by lower maternal control over feeding, predicted fewer maternal reports of negative interactions during mealtimes. An interesting point to consider here is that Farrow and Blissett (2006a) observed mothers feeding their

infants in their own homes. Most other published studies in this area rely on maternal report of feeding practices rather than more naturalistic methods of observation.

1.2.4.2 Maternal control during feeding

Parents influence their children's eating habits by determining the availability, preparation and quantity of food. Furthermore, eating behaviours of parents themselves also affect those of their children (Wardle, Carnell & Cook, 2005) through behavioural modelling and exposing children to certain foods. Parental control over children's feeding behaviours is an important factor that is implicated in children's food choice. Parent-centred (more controlling) feeding behaviours during childhood are associated with lower fruit and vegetable intake, whereas child-centred (more encouraging) feeding behaviours are associated with higher fruit and vegetable intakes in university students (Murashima, Hoerr, Hughes, Kattelman & Phillips, 2012).

Overly controlling feeding practices are also associated with: decreased self-regulation of energy intake; decreased fruit and vegetable consumption; increased consumption of soda, flavoured drinks, fats sweets and sugar cereals; dietary restraint; and negative self-evaluation of eating (Arredondo et al., 2006; Birch & Fisher, 1996; Carper, Fisher & Birch, 2000; Fisher & Birch, 2000; Fisher, Birch, Smiciklas-Wright, Picciano, 2000; Fisher, Mitchell, Smiciklas-Wright & Birch, 2002; Johnson & Birch, 1994). Control over feeding has been shown to vary according to socioeconomic status (Hupkens, Knibble, Van Otterloo & Drop, 1998; Kröller &

Warschburger, 2008), culture (Stearns, 1997) and gender of the child (O'Dea, 1999). It is also important to note that, the controlling strategies used by mothers have been described during both feeding and non-feeding interactions (Chatoor, Egan, Getson, Menvielle & O'Donnell, 1988).

Birch et al., (2001) distinguished two characteristics of parental control, pressure to eat and restriction. Pressuring children to eat refers to attempts to increase consumption of healthy foods and has been found to be more common in mothers who breastfeed for shorter durations or not at all (DiSantis et al., 2013). Fisher et al., (2002) found that pressuring feeding practices may have a negative impact on children's fruit and vegetable consumption. Crouch, O'Dea and Battisti (2007) found that mothers demonstrated greater degrees of parental control over obese than normal-weight children and that mothers who self-report the use of restrictive feeding practices also monitor their children's intake and use pressuring feeding practices also. Although causality cannot be inferred, it was proposed that mothers seem to restrict access to foods when they are worried over their child's weight.

Food restriction refers to limiting access to foods such as sweets and snacks high in fat. Restrictive feeding practices have been linked to: increased preference for and preoccupation with the food being restricted (Birch, Zimmerman & Hind, 1980; Ogden, Cordey, Cutler & Thomas, 2013); increased snacking behaviour (Birch & Fisher, 2000; Fisher & Birch, 1999); and higher body fat in children (Spruijt-Metz, Lindquist, Birch, Fisher & Goran, 2002). Furthermore, Kröller, Jahnke and Warschburger (2013) investigated the effects of feeding practices in 482 mothers on

emotional eating in their children. Mothers reported both their feeding practices and their child's eating behaviour. It was found that greater maternal restriction of food or food monitoring was related to greater emotional eating in children; allowing children more control over eating however, was associated with less pronounced emotional eating. Davison and Birch (2001) suggest that restriction of foods (usually energy-dense foods) may increase children's risk of overweight, as these children are likely to overeat the restricted foods when they have access to them. However, it is of importance to note here, that maternal feeding practices have been found to become more controlling after excessive rates of weight gain, not before (Rhee et al., 2009). Faith et al., (2004) found that among children predisposed to obesity, increased weight evokes restrictive feeding practices, which contradictory to intention, may lead to further weight gain.

In a study investigating effects of parental control over eating on inhibitory control in children, Johnson and Birch (1994) found that mothers whom exerted greater controlling feeding practices had children who were less able to self-regulate their energy intake. It was also found that children who had poorer self-regulation had greater body fat. It was concluded that the best environment for children to develop good self-control of their energy intake is one in which parents provide healthy food and then allow their children to decide and control how much they eat. It is important to consider here however, that although ability to adjust food intake was observed, maternal control during feeding was not. Mothers reported control via questionnaire. A preferable method would have been to observe a feeding interaction between the

mother and child. This way, both maternal feeding and child eating behaviour during a mealtime could have been quantitatively observed and analysed.

Anzman and Birch (2009), explored the effects of parental restriction on inhibitory control and weight status. Results revealed that girls who perceived greater parental restriction demonstrated the strongest negative association between inhibitory control and weight status; thus highlighting the importance of studying how controlling feeding practices may be perceived by the infant/child in addition to how they are reported by the parent. Future investigations should therefore seek to observe parental feeding practices and compare and contrast these observed behaviours to those self-reported by the parents. This will allow researchers to better investigate the effect parental control has on the development of eating behaviours and weight gain in children.

Moreover, Farrow and Blissett (2006b) investigated the effect of controlling feeding practices as a moderator of the association between early and later weight gain in the first 6-months of life. Results revealed that high maternal control was associated with further decelerated weight gain in infants who already demonstrated slow early weight gain and further accelerated weight gain in those who already demonstrated greater early weight gain. Moderate or low control was associated with the opposite pattern, accelerating weight gain in those who had demonstrated slow early weight gain, and vice versa. It is important to consider here however, that although controlling feeding strategies appear to exacerbate certain eating behaviours and

weight gain trajectories, these problems themselves may just as easily provoke the controlling strategies to enforce/restrict food consumption (Douglas, 2000).

From the literature discussed thus far, it can be seen that breastfeeding, in conjunction with greater maternal sensitivity and lower control, confer a more general protective effect over healthful eating behaviours and patterns of weight gain in infants. A natural progression of this research would be to begin looking at the interaction of these factors along with biological predictors of adiposity, particularly those present in breast milk. This way, more detailed investigation into the predictors of weight gain and development of obesogenic eating behaviours in infancy can be undertaken.

1.3 Cortisol, eating and weight

1.3.1 Cortisol level, eating behaviours and weight

One potentially important biologically active ingredient of breast milk that has implications for eating behaviours and weight gain is cortisol. Cortisol is a glucocorticoid; a main function of which is to maintain normal blood glucose levels and help store excess glucose for future use. Cortisol is present in blood, urine and saliva (Cao et al., 2009), and has been related to obesogenic eating behaviours and overweight in human adults (Björntorp & Rosmond, 2000; Dallman et al., 2003; Marin et al., 1992; Pasquali et al., 1993; Tataranni et al., 1996). Patacchioli et al., (1992) investigated the presence of cortisol in breast milk and found that the act of breastfeeding did not affect maternal cortisol levels in the first 3-days postpartum. It

was also found that maternal cortisol levels in plasma and breast milk were strongly correlated. These findings therefore suggest that the level of cortisol in plasma can reliably predict the level of cortisol in breast milk.

Glucocorticoids are also part of the hypothalamic-pituitary-adrenal (HPA) stress response and are released to help us cope with stressful experiences. The HPA axis refers to a system of feedback interactions between the hypothalamus, pituitary gland and the adrenal glands. It controls reactions to stress and is involved in the regulation of body temperature, digestion, immune system, mood, emotions, sexuality and energy usage. When an environmental stressor is encountered, neurons in the hypothalamus are triggered to release the hormones corticotrophin-releasing hormone (CRH) and arginine-vasopressin (AVP). CRH stimulates the release of corticotrophin, which then triggers the production of cortisol. Vasopressin increases blood pressure by increasing reabsorption of water by the kidneys and contraction of blood vessels. Together, CRH and vasopressin activate the HPA axis. It is also of interest to note here, that in obesity, pituitary response to CRH/AVP stimulation appears to be altered. Furthermore, this seems to be predominantly related to the individual's body size and total body fat. Hyper-responsiveness to ACTH after CRH/AVP stimulation also appears to be related to hyperinsulinaemia – a condition associated with obesity where excess levels of insulin circulate in the blood (Pasquali et al., 1999).

Cortisol regulates blood glucose levels by: (1) stimulating gluconeogenesis, increasing glucose production; and (2) stimulating hepatic glycogen synthesis.

Cortisol is part of the inhibitory feedback loop and when cortisol levels are increased, secretion of CRH is blocked thus preventing HPA axis interactions and further secretion of cortisol (Randall, 2011). Taking this into account, quicker cortisol recovery, after a stressor, is therefore indicative of a better stress response.

When cortisol levels are persistently elevated, several systems begin to show pathological changes including the immune system and organ function. One of the pathological changes associated with persistently elevated cortisol levels in human adults is neuronal atrophy in the hippocampus (Sapolsky, 1996). The hippocampus helps shut off the HPA response to stress; therefore hippocampal damage impairs this, resulting in a prolonged stress response and elevated levels of glucocorticoids in the body (Herman & Cullinan, 1997; Jacobson & Sapolsky, 1991). As elevated cortisol levels have been found to increase caloric intake in human adults (Tataranni et al., 1996), damage to the hippocampus may lead to long-term overconsumption.

Cortisol is believed to be involved in the regulation of appetite (Wolkowitz, Epel & Rues, 2001) and energy balance by increasing energy intake in response to stress (Epel, Lapidus, McEwen & Brownell, 2001). Dallman et al., (2003) proposed that the rise in cortisol in response to stress may cause overeating of comfort foods which may then lead to obesity. In support of this, Francis, Granger and Susman (2013) found that changes in cortisol release in response to psychological stress was related to higher BMI and increased eating in the absence of hunger in 8- to 9-year-old children. Epel et al., (2001) exposed human adult participants to a stress condition and a control condition (on different days). It was revealed that participants

categorised as high cortisol reactors ate more calories on the stress day compared to low cortisol reactors and the rise in negative mood in reaction to stress was associated with larger caloric consumption. Both groups ate similar amounts on the control day. In addition to this, high cortisol reactors ate significantly more sweet foods over both days.

Newman, O'Connor and Connor (2007) aimed to find out if the relationship between hassles and snacking outside of the laboratory differed between participants high and low in cortisol reactivity. Fifty women underwent a stressor in the lab, provided saliva samples to assess cortisol reactivity, then completed daily hassles and snack intake diaries for 14-days. It was found that increased daily hassles were significantly related to increased snacking in high cortisol reactors but not low cortisol reactors. Furthermore, across the whole sample, snack intake was significantly related to dietary restraint, emotional eating, disinhibition and external eating. It is of interest to note that all associations were stronger amongst high cortisol reactors, suggesting that cortisol reactivity may explain, at least in part, the moderating role of eating style on stress-induced eating (Newman et al., 2007). The results of this research therefore suggest that the psychophysiological response to stress may influence subsequent eating behaviour.

In addition to the effect of endogenous glucocorticoids, exogenous glucocorticoids have also been found to influence eating behaviours. Uddén et al., (2003) treated 12 obese women with glucocorticoids for 7-days and found that food intake significantly increased by 20% after treatment. Tataranni et al., (1996) gave either glucocorticoid

or placebo to human adult males and found that administration of glucocorticoids increased energy consumption significantly more than the placebo treatment, especially intakes of carbohydrates and proteins. The authors suggested that therapeutic doses of glucocorticoids cause obesity through increases energy intake and this may be due to a direct or indirect action of glucocorticoids on the central regulation of appetite (Tataranni et al., 1996).

The proposed association between cortisol level and overweight has been widely researched and it is suggested that chronic stress promotes insulin release, which then leads to the activation of abdominal fat storage (Dallman, Akana, Strack, Hanson & Sebastian, 1995). Persistently raised levels of cortisol lead to excessive increases of, and then resistance to, insulin and leptin (Björntorp, 2001; Björntorp & Rosmond, 2000) and abdominal obesity (Björntorp & Rosmond, 2000). Therefore, as illustrated in Figure 1.2, elevated cortisol levels induced by stress could lead to reduced sensitization of satiety signals and reduced ability to respond to excess weight gain (Adam & Epel, 2007). The diagram also illustrates that increased cortisol exposure has both direct and indirect effects on the reward system, heightening its sensitization, which in turn can lead to greater consumption of highly palatable food. Therefore, the combination of elevated cortisol, dense calories, and excessively high insulin, contributes to fat distribution (Adam & Epel, 2007).

It is of importance, here, to acknowledge research that has investigated effects of oxytocin on hedonic eating. Oxytocin is a hormone known to be released during labour and during breastfeeding; it regulates physiological functions related to

reproduction and mother-infant interaction (Meyer-Lindenberg, Domes, Kirsch, & Heinrichs, 2011). However, research using animal models suggests it also inhibits food intake and affects glucose homeostasis and energy expenditure (Morton et al., 2012; Olson et al., 1991).

Ott et al., (2013) found that oxytocin, in addition to its effects on human bonding and interaction does in fact regulate reward-related food intake. More specifically, intranasal oxytocin was found to markedly reduce snack consumption (particularly chocolate cookies). Oxytocin reduced levels of adrenocorticotrophic hormone and cortisol and limited the rise in plasma glucose associated with consumption of a meal. Interestingly, although oxytocin appeared to regulate reward-related snack intake, it did not affect food intake driven by hunger (Ott et al., 2013). According to the literature, it is therefore possible that although elevated cortisol, dense calories, and excessively high insulin, contribute to fat distribution (Adam & Epel, 2007), increasing an individual's exposure to hormones with anorexigenic effects (such as oxytocin) may help reduce cortisol level and hedonic snack intake.

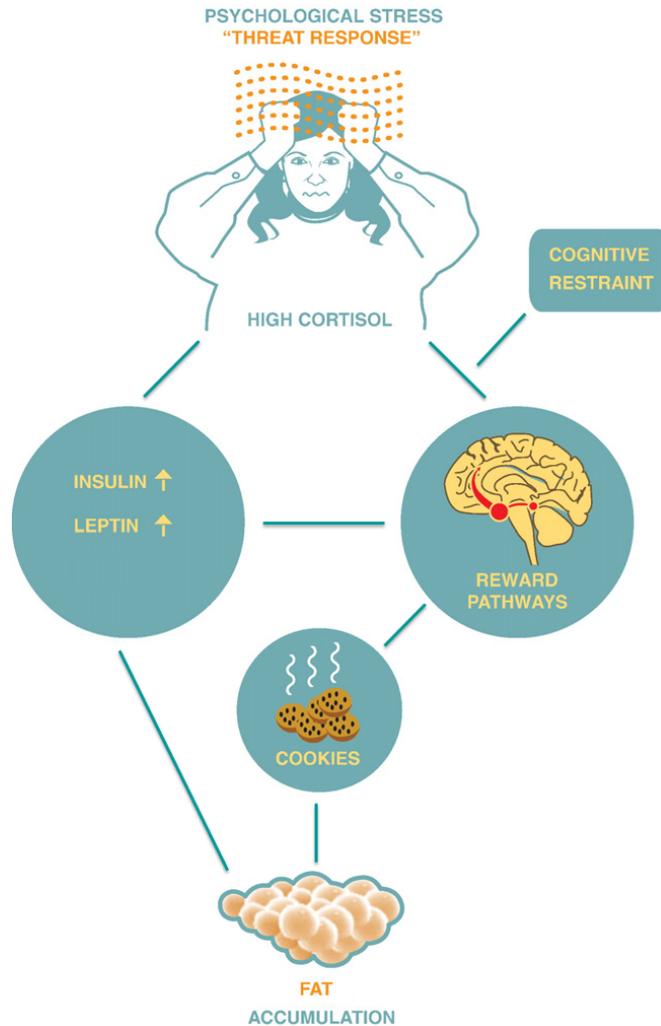


Figure 1.2

Model illustrating effects of high cortisol on eating behaviours and fat accumulation (adapted from Adam & Epel, 2007).

Initial studies treated animals in the laboratory with glucocorticoids and found that this results in obesity; obese animals also demonstrate higher concentrations of corticosterone, a main glucocorticoid in animals (York & Bray, 1972). In addition to this, removal of the adrenal glands, which synthesise glucocorticoids, prevents obesity and its related metabolic changes in rat models (Bray, 1982; Freedman, Horwitz & Stern, 1986). Unfortunately, the role of glucocorticoids in humans is less well understood. The more convincing evidence of the relationship between cortisol

level and obesity is derived from research that distinguishes between central and peripheral types of obesity. For example, serum and urinary concentrations of cortisol have been found to increase with greater waist/hip ratio and abdominal diameter in obese women (Marin et al., 1992). Furthermore, serum cortisol response to stress is more pronounced in women with higher waist/hip ratios (Marin et al., 1992; Pasquali et al., 1993), therefore highlighting a possible role of cortisol response to environmental stress in abdominal obesity (Björntorp, 1995).

Contradictory to the aforementioned research however, more recent studies have demonstrated that in obese individuals, although cortisol secretion is elevated, the circulatory concentrations are normal or low (Björntorp & Rosmond, 2000). These normal, or even low, plasma concentrations of cortisol have been explained due to an enhanced excretion of cortisol accompanied by an increased metabolic clearance rate of it (Andrew, Phillips & Walker, 1988). This was supported by Vierhapper, Nowotny and Waldhäusl (2004), who found that metabolic clearance rates of cortisol were significantly higher in obese, than non-obese, women although production rates were similar for both groups. Indeed, evidence suggests that the overall level of serum cortisol may not be as important as the variations in cortisol metabolism in the investigation of weight and adiposity. Changes in the cortisol metabolism result in variable active glucocorticoids within the body; this highlights the importance of the investigation of the *metabolism* of cortisol in the context of eating behaviour and obesity.

1.3.2 Cortisol metabolism

The enzymes responsible for the interconversion of cortisol and cortisone are 11 β -hydroxysteroid dehydrogenase types 1 and 2 (11 β HSD1 and 11 β HSD2). As pictured in Figure 1.3, 11 β HSD1 converts inactive cortisone into active cortisol and 11 β HSD2 inactivates cortisol into cortisone (Funder, 1985). 11 β HSD2 is located in mineralcorticoid tissues such as the kidney and the placenta; 11 β HSD1 is located in glucocorticoid tissues including liver, adipose and muscle (Tomlinson et al., 2007). Another important enzyme involved in steroid metabolism is 5- α reductase (5 α -reductase; an A-ring reductase), which enhances cortisol clearance by breaking it down into tetrahydro-metabolites (Raffaelli, Sabaa, Vignalib, Marcoccib & Salvadori, 2006). 5 α -reductase is expressed in skin, adipose tissue and liver (Russell & Wilson, 1994).

It is important to note here that currently, there is no normative data on cortisol metabolism in human infants. In fact, the developmental aspects of glucocorticoid metabolism in general require further examination (Dötsch, Hohenberger, Peter, Sippell & Dörr, 2000). Research has found significant associations between maternal and infant cortisol levels at 6- and 12-months postpartum (Stenius et al., 2008; Yehuda et al., 2005) but there is no published research investigating relationships between maternal and infant cortisol metabolism. It is of particular interest to examine cortisol metabolism in the first year of life, because early glucocorticoid metabolism is poorly understood, although it does appear to change at approximately 3-months of age (Dötsch et al., 2000), when infants are able to convert cortisone to

cortisol. It is possible that individual variation in the development of early cortisol metabolism may be linked to variation in weight status in the first year of life.

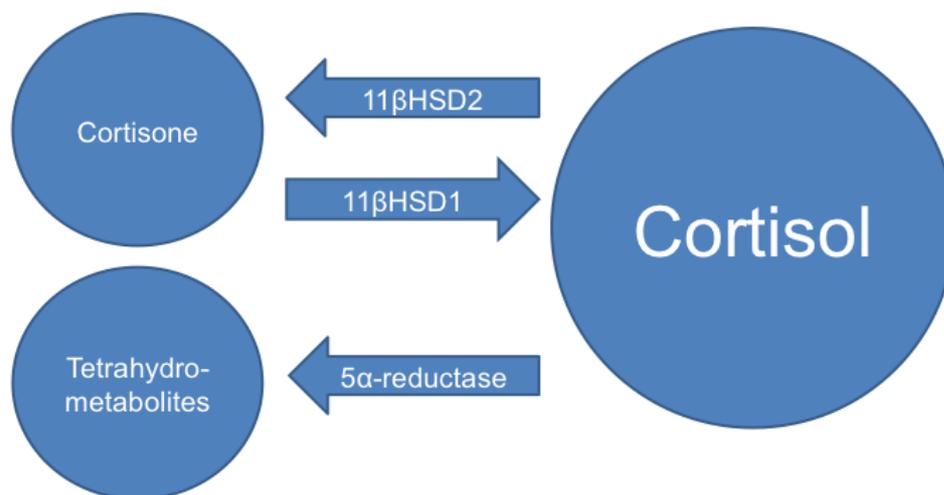


Figure 1.3

Enzymes involved in the metabolism of cortisol

1.3.3 Cortisol metabolism and weight

Activity of 11 β HSD1 has been found to be dysregulated in obesity (Stewart, Boulton, Kumar, Clark & Shackleton, 1999). In recent years, numerous studies have been published that examine 11 β HSD1 expression in human adipose tissue. Rask et al., (2001) found that activation of cortisone to cortisol by 11 β HSD1 in the liver is impaired in adults with obesity. It was also discovered that changes in 11 β HSD1 in obesity are tissue specific and activation of cortisol in subcutaneous adipose tissue may exacerbate obesity. Rask et al., (2001) suggested that inhibiting 11 β HSD1 in the adipose tissue of obese patients may be beneficial as it promotes increased insulin sensitivity and restricts weight gain in obesity. Studies examining 11 β HSD1 activity in subcutaneous adipose tissue have also found positive correlations with BMI and

insulin resistance (e.g. Kannisto et al., 2004). Lindsay et al., (2003) found that 11 β HSD1 activity in subcutaneous adipose tissue was positively related to measures of BMI, percentage body fat and waist circumference. It was concluded that increased regeneration of cortisol in adipose tissue influences metabolic development of human obesity.

Research has also investigated 11 β HSD1 activity in visceral adipose tissue. These studies report similar findings as those looking at subcutaneous adipose tissue (e.g. Veilleux, Rheaume, Daris, Luu-The & Tchernof, 2009). Baudrand et al., (2010) found that in a sample of morbidly obese patients, 11 β HSD1 expression is: significantly higher in the liver than in subcutaneous or visceral adipose tissue; and significantly greater in subcutaneous than visceral adipose tissue. In addition to this, there were no gender differences in 11 β HSD1 levels between liver, subcutaneous or visceral adipose tissue. Paulsen, Pedersen, Fisker and Richelsen (2007) found that 11 β HSD1 in subcutaneous and visceral adipose tissue was higher in obese compared with lean participants. Although this study did not find a difference between obese male and female participants, lean females were found to demonstrate 62% lower 11 β HSD1 expression than lean males in subcutaneous adipose tissue. Unlike Baudrand et al., (2010), Paulsen et al., (2007) found no difference in mRNA expression of 11 β HSD1 between visceral and subcutaneous adipose tissue in both lean and obese participants. Results from Baudrand et al., (2010) and Paulsen et al., (2007) therefore are inconsistent regarding whether or not 11 β HSD1 is higher in subcutaneous than visceral adipose tissue. Results from Paulsen et al., (2007) indicate that lean women activate less cortisone into cortisol in

subcutaneous adipose tissue than obese women, although it cannot be inferred from this whether or not this results in higher circulating cortisol.

Inactivation of cortisol by 5 α -reductase is increased in obese men and women (Andrew, Phillips & Walker, 1998) and is positively associated with BMI in obese and non-obese women (Vassiliadi et al., 2009) and insulin resistance in obese men and women (Tomlinson et al., 2008). This increased inactivation of cortisol in human obesity occurs alongside a reduction in cortisol activation via 11 β HSD1 and represents a compensatory mechanism to enhance the clearance of cortisol and therefore improve insulin sensitivity (Tomlinson et al., 2008). In addition to this, Baudrand et al., (2011) found that there is an over-expression of 5 α -reductase in the liver of morbidly obese male and female human adults and that this over-expression may have a protective effect in preserving insulin sensitivity in the liver.

Müssig et al., (2008) investigated glucocorticoid metabolism in men and women and although enzyme activity of 5 α -reductase and 11 β -HSD1 were similar in lean and obese individuals, 11 β -HSD2 activity was distinctly elevated in adiposity. Furthermore, 11 β -HSD2 activity was significantly associated with insulin sensitivity. It was concluded that in obese men and women, the kidney may increase its supply of cortisone for 11 β -HSD1 (outside of the kidney), and this may fuel visceral adiposity and insulin resistance. Although there is no published research investigating cortisol metabolism and infant weight gain, there is evidence that exposure to exogenous and endogenous glucocorticoids in utero is associated with low birth weight (Braun et al., 2013; Murphy et al., 2002; Stewart, Rogerson & Mason, 1995).

The aforementioned research demonstrates that weight and obesity has important implications for cortisol metabolism in human adults; however there are currently no studies examining the role of cortisol metabolism in weight gain in infancy. What's more, although there are studies that have investigated cortisol level and reactivity on eating behaviours, there is no research examining the relationships between cortisol metabolism and eating behaviours in human adults or infants. Early relationships between cortisol metabolism and infant adiposity are therefore unknown.

1.3.4 Prenatal influences of corticosteroids on growth

Research has shown that adverse influences during early development, especially prenatally, can result in permanent changes in physiology and metabolism of the infant; these changes have long-term consequences on health and disease throughout childhood, adolescence and into adulthood. Low birth weight is considered to be a marker of an adverse intrauterine environment and has been found to be associated with increased mortality and a range of metabolic disorders such as insulin resistance, type 2 diabetes and obesity, as well as hypertension and death from cardiovascular disease (Barker, 1995). One of the key mechanisms involved in this association may be an over-exposure of the developing foetus to glucocorticoids, which can be caused by placental and/or maternal factors such as stress, malnutrition and disease (Reynolds, 2010).

Research has shown that intrauterine exposure to exogenous and endogenous glucocorticoids is associated with low birth weight and low birth weight is related to

increased risk of abdominal adiposity between 6- and 13- (Jaiswal et al., 2012) and at 23-years of age (Atkinson, Steele, Stoskoff, & Saigal, 2005). For example, it has been found that lower activity of placental 11 β -HSD2 is associated with lower birth weight in human infants (Murphy et al., 2002; Stewart et al., 1995), which suggests that greater exposure to maternal cortisol in utero has an important effect on later development (Reynolds, 2010). In addition to this, eating high quantities (≥ 500 mg/week) of liquorice, which inhibits 11 β -HSD2, whilst pregnant is associated with giving birth prematurely to a smaller infant (Strandberg, Andersson, Jarvenpaa, McKeigue, 2002). Furthermore, treating women at high risk for premature delivery with glucocorticoids has also been associated with reduced foetal growth in human infants as demonstrated through lower birth weight, body length and head circumference (Braun et al., 2013).

It is important to also recognise evidence that has not found a relationship between low birth weight and later adiposity. For example, Ma et al., (2012) found that infants who were overweight at 12-months-old had higher birth weights and had gained significantly more weight over the first year than infants who were of normal weight at 12-months. However, in overweight infants, parental report of feeding infants quickly and feeding to resolve infant fussiness were positively associated with BMI-for-age z-scores (Ma et al., 2012), suggesting parental feeding practices must be considered also. Furthermore, Goodell, Wakefield and Ferris (2009) found that it was not low birth weight, but rapid weight gain during the first year of life that predicted obesity in 2- to 3-year-olds. In support of this, a systematic review by Baird et al., (2005)

concluded that infants who grew more rapidly, irrespective of birth weight, were more likely than other infants, to be obese in childhood, adolescence, and early adulthood. Published findings clearly highlight the implications of being exposed to excessive levels of glucocorticoids in utero in terms of later weight and metabolic disease. These findings reinforce the importance of investigating effects of cortisol metabolism and early infant weight gain so that early relationships between cortisol metabolism and infant adiposity can be better understood.

1.3.5 Mechanisms underlying the relationship between cortisol, eating and weight

There are several mechanisms, which may underlie the relationship between cortisol, eating and weight in humans. Spencer and Tilbrook (2011) conducted a review which discussed several ways in which glucocorticoids may be involved in appetite regulation, adiposity and metabolism. Firstly, the authors explained that glucocorticoids stimulate food consumption through their direct action on orexigenic NPY/AGRP neurons in the hypothalamic arcuate nucleus. Secondly, glucocorticoids also reinforce the rewarding nature of food by stimulating the nucleus accumbens (NAcc)/VTA pathways. The review also describes that increased glucocorticoids are associated with upregulated ghrelin signalling and may stimulate food consumption by enhancing the appetite- and reward-stimulatory effects of ghrelin.

Furthermore, Spencer and Tilbrook (2011) also explain that glucocorticoids can prevent the actions of insulin and leptin, which are two hormones that suppress

appetite. Preventing the action of these hormones reduces the brain's sensitivity to insulin and leptin and eventually contributes to the development of insulin and leptin resistance. Finally, the authors describe that glucocorticoids also contribute to enhancing visceral fat at white adipose tissue; glucocorticoids activate hormone-sensitive lipase (which enhances lipolysis) and lipoprotein lipase (which promotes fat storage). Therefore, the greater density of glucocorticoid receptors in visceral fat, combined with the fact that there is more lipoprotein lipase activity, means that fat storage may be increased in visceral adipose tissue in the presence of increased glucocorticoids.

Regarding the relationship between the metabolism of cortisol and weight, it has been argued that elevated 11β -HSD1 is a cause, rather than consequence, of weight gain and obesity, as inhibiting it improves metabolism and reduces weight (Tiwari, 2010). In addition to this, research conducted by Morton et al., (2004) has shown that disrupting 11β -HSD1 is protective of weight gain that is associated with high-fat diets. However, although research clearly demonstrates that glucocorticoid activity is related to adiposity and weight in human adults, and being exposed to excessive levels of glucocorticoids in utero affects later weight and metabolic disease, the mechanisms by which this occurs are unknown (Spencer & Tilbrook, 2011).

1.4 Cortisol, breastfeeding and maternal sensitivity

1.4.1 Cortisol and breastfeeding

In addition to eating behaviours and overweight, cortisol has also been researched with regard to breastfeeding. It has been previously argued that less skin-to-skin contact from the mother, as with formula-feeding for example, is a powerful stressor to infants (Harlow & Suomi, 1970). In addition to this, breastfeeding has been found to evoke a palliative effect (Carbajal, Veerapen, Couderc, Jugie & Ville, 2003). It may then be reasonable to assume that breastfed infants have lower levels of cortisol than their formula-fed counterparts due to increased contact and comfort through being breastfed.

Cao et al., (2009) however, found that breastfed infants in fact demonstrated salivary cortisol levels 40% higher than those of formula-fed infants throughout the first year of life. It was suggested that breastfeeding requires more physical effort by the infant than formula-feeding and it might be this increased effort that partly explains the higher cortisol levels observed in breastfed infants. As cortisol during infancy has an important role in the development of the infant's HPA axis and stress response, results of this study suggest that the elevated levels of cortisol in breastfed infants may have a programming effect on the developing HPA axis.

Beijers, Riksen-Walraven and de Weerth (2013) investigated effects of breastfeeding duration on cortisol regulation in 193 infants involved in a wider longitudinal study investigating early caregiving and child development. Breastfeeding information was

collected weekly during the first 6-months postpartum and at 12-months infants completed the Strange Situation Procedure (Ainsworth et al., 1978) in order to induce stress. Salivary cortisol was measured pre-stressor and also at 25- 40- and 60- minutes post-stressor to measure cortisol reactivity and recovery from stress. It was found that a longer duration of breastfeeding predicted faster cortisol recovery. Interestingly, these results were found after controlling for effects of maternal sensitivity, depression, education, infant gender and attachment status, amongst other cofounders. The authors concluded that breastfeeding positively contributes to infant cortisol regulation. These results therefore suggest that a longer duration of breastfeeding may contribute to the programming of the HPA axis during infancy.

It is also important to consider research by Mezzacappa and Katkin (2002) who found that breastfeeding mothers reported less stress than mothers who were formula-feeding. Breastfeeding was related to a reduction in negative maternal mood and formula feeding was related to a reduction in positive mood from pre- to post-feeding. It has also been found that although 10-minutes after breastfeeding, mothers' systolic blood pressure is elevated (Mezzacappa, Kelsey, Myres & Katkin, 2001), one hour after feeding, breastfeeding mothers had lower blood pressures than mothers who fed their infants formula (Light et al., 2000). These results suggest that breastfeeding has an anti-stress effect on the mother, which may in turn have a soothing effect on the infant.

Hart et al., (2004) found that the concentration of cortisol in maternal breast milk predicted 'better' performance on the Autonomic Stability cluster of the Neonatal

Behavioural Assessment Scale (NBAS). Cortisol in breast milk was positively related to homeostatic adjustments of the infant's central nervous system and improved capabilities to control involuntary responses (including tremors, startles and skin colour changes). This is very important as the relationship between breast milk cortisol and superior behaviour of infants (demonstrated through optimised arousal and higher NBAS score) may relate to their own plasma cortisol levels (Gunner, Isensee, & Gust, 1987; Magnano, Garder, & Karmel, 1992). This suggests that maternal cortisol may influence infant cortisol and behaviour in the pre- and/or postnatal period. Prenatally this will be related to how much cortisol can pass through the placenta; postnatally it is possible that infant cortisol and behaviour may be influenced through the level of maternal cortisol present in breast milk. Infant cortisol and behaviour may also be mediated by sensitivity of maternal behaviour (see Figure 1.4).

Although breastfeeding has been found not to affect maternal cortisol levels in the immediate postnatal period (Patacchioli et al., 1992), research investigating the long-term relationship between breastfeeding and maternal cortisol levels has shown the opposite effect. Lankarani-Fard, Kritz-Silverstein, Barrett-Connor and Goodman-Gruen (2001) found that mothers who breastfed for longer than 12-months have significantly higher cortisol levels between the ages of 50- and 89-years, than those who breastfed for shorter durations or not at all. The authors suggested that breastfeeding may have a protective role against some auto-immune diseases and this effect may be mediated by cortisol (Lankarani-Fard, et al., 2001). Furthermore, research has also shown that mothers with higher levels of cortisol have increased

accuracy in identifying their infants' odours, enhanced preference for these odours and show more affection to their infants (Fleming, Steiner & Anderson, 1987; Fleming, Steiner & Corter, 1997). These findings suggest that maternal cortisol may be involved in maternal sensitivity to infant cues. The behaviour displayed by infants may therefore be a result of greater maternal sensitivity and/or the level of cortisol to which the infant is exposed to (see Figure 1.4).

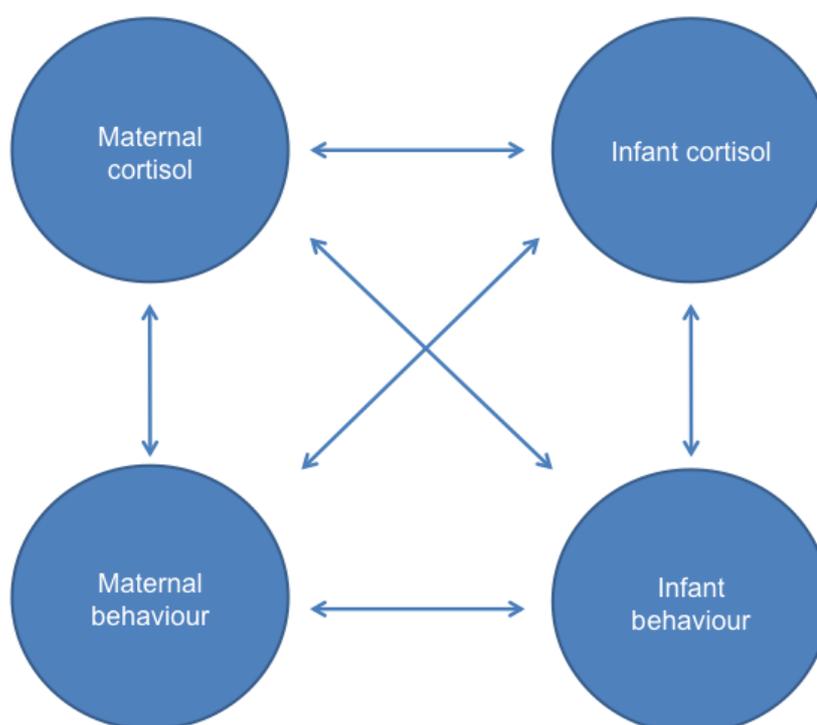


Figure 1.4

Model illustrating relationships between infant and maternal cortisol and behaviour

It is possible that variations in infants' own cortisol metabolism may be linked to environmental variables such as breastfeeding or maternal sensitivity, yielding future potential intervention strategies for the prevention of early risk factors for obesity. However, there is no current research investigating interactions between maternal sensitivity, infant cortisol metabolism and the quality of mother-infant feeding

interactions throughout the first postnatal year. Given that cortisol is present in breast milk and infant cortisol may vary according to maternal sensitivity, a potentially fruitful investigation would be to examine the links between formula-feeding, breastfeeding, cortisol metabolism and maternal sensitivity in the prediction of eating behaviours and weight gain in infancy.

1.4.2 Cortisol and maternal sensitivity

A final justification for exploring the relationships between the metabolism of cortisol and maternal sensitivity in this context is that cortisol disturbances are associated with a variety of psychological and behavioural disturbances in humans. Animal models have demonstrated that early maternal care has a functional effect on the HPA axis in infants, suggesting that maternal behaviour may have an organisational effect on the infant's stress regulation responses and glucose regulation (Murray, Halligan, Goodyer & Herbert, 2010).

When examining the effect of maternal anxiety and depression on infant cortisol production, it has been found that mothers who have both anxiety and depression have infants who produce more cortisol from morning to evening compared to mothers with depression only or no depression at all (Azak, Murison, Wentzel-Larsen, Smith & Gunnar, 2013). Human models have shown that the 9-month-old infants of depressed mothers have higher cortisol reactivity and less mature regulatory behaviours than the infants of non-depressed mothers (Feldman et al., 2009). Furthermore, maternal anxiety and maternal insensitivity have been shown to

be additive, independent predictors of 7-month-old infants' cortisol reactivity in response to a stressful situation (Grant et al., 2009).

These results initially appear contradictory to aforementioned findings that: (1) increased cortisol in breast milk is predictive of infants demonstrating better homeostatic adjustments of the central nervous system and improved control of involuntary responses (Hart et al., 2004); and (2) mothers with increased cortisol levels show an increased accuracy in identifying their infants' odours, enhanced preference for these odours and show more affection to their infants. It is possible however, that as cortisol is essential for life, there is an optimal level of cortisol that is necessary and adaptive for human function. It is known that persistently elevated levels of cortisol are associated with pathological changes to the immune system and organ function (Sapolsky, 1996). Therefore it is expected that levels of cortisol that are both too high, and too low, have negative consequences on human behaviour.

Longitudinal research has also suggested that maternal withdrawal during the first postnatal year has long-term implications for the HPA axis; for example, withdrawn parenting, seen in mothers with depression in the first 9-months postpartum, is associated with elevated cortisol secretion in 13-year-old offspring (Murray et al., 2010). In summary, cortisol level has been linked to behavioural problems, sensitivity and mental health in both adults and children (Ashman, Dawson, Panagiotides, Yamada & Wilkinson, 2002; Biederman et al., 1993; Essex, Klein, Cho & Kalin, 2002; Essex, Klein, Slattery, Goldsmith & Kalin, 2010; Kagan, Reznick & Snidman, 1988; McBurnett, Lahey, Rathouz & Loeber, 2000; Rosenblitt, Soler, Johnson & Quadagno,

2001; Schmidt et al., 1997; Smider et al., 2002; van Goozen et al., 1998). However, no studies have yet considered the relationship between cortisol metabolism and less adaptive feeding behaviours in infancy and childhood, nor the potential involvement of breastfeeding in this relationship. Maternal behaviour towards an infant has been described as 'an organiser of psychobiological function in the first year of life' (Spangler, Schieche, Ilg, Maier & Ackermann, 1994). Therefore the interactions between maternal behaviour, feeding practices, infant cortisol metabolism and growth warrant further examination.

1.5 Demographic and psychological factors

A variety of demographic and psychological factors have been related to infant feeding outcomes, maternal sensitivity and infant weight gain. Any study in this research area therefore needs to measure and consider these factors. Maternal weight, restrained eating, body dissatisfaction, history of eating disorder psychopathology and mental health problems have all been found to predict maternal feeding practices which in turn predict the eating behaviours and weight gain of their children (Birch & Fisher, 2000; Duke, Bryson, Hammer & Agras, 2004; Farrow & Blissett, 2005; Farrow & Blissett, 2009).

Farrow and Blissett (2005) investigated relationships between symptoms of maternal psychopathology and feeding practices in 87-women and their 1-year-old infants. It was found that anxious psychopathology, as reported by mothers during pregnancy and at 6- and 12-months postpartum, predicted the use of negative restrictive feeding

practices. This relationship was significant even when breastfeeding duration was accounted for. An important strength to note of studies such as this is their longitudinal nature. Longitudinal data is vital in increasing our understanding of factors that influence the development of eating behaviours and patterns of weight gain.

Parental obesity arguably has the strongest influence on childhood obesity (Parsons, Power, Logan & Summerbell, 1999). Li, Law, Lo Conte and Power (2009) found that children and adolescents were more likely to have a high BMI and be at risk from obesity if their parents had demonstrated excessive BMI gain in their own childhood and adulthood. This could be due to both genetic components and a shared environment. It is also important to note here that overweight and obese mothers are less likely to initiate and maintain breastfeeding, and exclusively breastfeed their infants, than women within a normal BMI range (Amir & Donath, 2007; Hilson, Rasmussen & Kjolhede, 1997; Li, Jewell & Grummer-Strawn, 2003).

There are differences across the studies that have attempted to control for maternal weight in terms of when weight was reported; the maternal weight or BMI that is reported may relate to any of the following time points: pre-pregnancy, the beginning of pregnancy, less than 1-year after delivery or greater than 1-year after delivery. Few studies report measuring maternal height and weight (e.g., Araujo, Victoria, Hallal & Gigante, 2006; Buyken et al., 2008; Karaolis-Danckert, Gunther, Kroke, Hornberg & Buyken, 2007); and most studies have relied upon maternal self-report of

this information through questionnaires and interviews (e.g., Burdette, Whitaker, Hall & Daniels, 2006; Huus, Ludvigsson, Enskär & Ludvigsson, 2008).

Socioeconomic status is another variable that confounds the relationship between breastfeeding and obesity. Also, demographic factors related to the increased likelihood of using controlling feeding practices include maternal age and educational level attained (Taveras et al., 2004). Woo, Dolan, Morrow, Geraghty and Goodman (2008) found that adolescents without a university-educated parent were less likely to have been breastfed for longer than 4-months. The authors concluded that if the duration of breastfeeding could be increased, this may lead to lower adolescent adiposity and even reduce the socioeconomic disparities in adiposity. Socioeconomic status can be measured by parental education, profession and income (Arenz et al., 2004). However, there is variation across studies regarding how it is assessed; some studies do not mention any measure of socioeconomic status at all (e.g., Davis et al., 2007; Mayer-Davis et al., 2006; Miralles, Sánchez, Palou & Picó, 2006; Ong et al., 2006; Schaefer-Graf et al., 2006; Shehadeh, Weitzer-Kish, Shamir, Shihab & Weiss, 2008); whereas other studies consider parental education but not household income (e.g., Burke et al., 2005; Moschonis, Grammatikaki & Manios, 2008).

Smoking during pregnancy is another factor that is likely to confound the relationship between breastfeeding and obesity. Al Mamun et al., (2006) investigated this in a prospective birth cohort study of 3,253 14-year-olds. It was found that BMI and prevalence of overweight and obesity was higher in adolescents whose mothers had smoked during their pregnancy. Additionally, for those whose mothers had stopped

smoking in pregnancy but did smoke at other points in the child's life, the adolescents' BMI was similar to that of those whose mothers had never smoked. A meta-analysis of 14 studies by Oken, Levitan and Gillman (2008) also found that children had a 50% increased risk of becoming obese before reaching adolescence if their mothers smoked whilst they were pregnant. Furthermore, Toschke, Montgomery, Pfeiffer and von Kries (2003) analysed data on 4,974 German children and found that if mothers smoked during the first trimester of pregnancy, their children had an elevated risk of being overweight at 5- and 6-years-of-age. The authors of this study also found that obese mothers were more likely to smoke when pregnant than mothers who were not obese. With regards to breastfeeding, Haug et al., (1998) found that mothers who smoke are more likely to breastfeed for shorter durations or not at all. The authors suggested that this may be due to insufficient milk production. In support of this, Hill and Aldag (1996) found that women who smoke report stopping breastfeeding early due to having an insufficient amount of milk. For those mothers who smoke whilst breastfeeding, it has been found that their infants are at an increased risk of becoming overweight at 7-years-old when compared to infants of mothers who smoke and exclusively formula feed (Wen, Shenassa & Paradis, 2013).

Other factors related to maternal physical health are also associated with overweight and obesogenic eating behaviours in their offspring. Children are at an increased risk of obesity if their mothers had diabetes when pregnant (Schaefer-Graf et al., 2005). Gestational diabetes results in over-nutrition of the foetus as it is exposed to elevated levels of glucose, amino acids and fatty acids. It has been proposed that infants born

to diabetic mothers are larger at birth. Interestingly, in a study of 125 Canadian women and their children, breastfeeding was found to lower the risk of obesity in children whose mothers had pre-gestational diabetes (Feig, Lipscombe, Tomlinson & Blumer, 2010). The authors concluded that due to the increased risk of obesity in their children, diabetic mothers should be encouraged to breastfeed their infants to help reduce this risk (Feig et al., 2010). Furthermore, elevated cortisol has a causative role in the development of high blood pressure. It is therefore important to ascertain from participants, their medical history and current medication, as certain conditions and medications, particularly those containing steroids, have an effect on cortisol metabolism. For example, it is important to establish whether individuals suffer from asthma, as the treatment for this condition is often a steroid inhaler. The aforementioned demographic, psychological and physical measures were included as covariates in the current study.

There is a wide variety of demographic and psychological factors related to infant feeding outcomes and weight gain; those involved in breastfeeding, weight gain, eating behaviours and maternal sensitivity have been highlighted here. These factors include, maternal weight and BMI, educational level, household income and socioeconomic status, maternal smoking, particularly during pregnancy, and maternal psychopathology and medical problems such as gestational diabetes. It is therefore extremely important that any study investigating the development of infant eating behaviours and weight gain accounts for these variables.

1.6 Summary

It can be understood from the aforementioned research that the source of milk during feeding, controlling feeding practices, insensitive communication and negative interaction all contribute to the likelihood of developing obesogenic eating behaviours and overweight in childhood, and that one potential explanatory mechanism by which this may occur is through the effects of these factors on, and individual variation in, cortisol metabolism. However, research is yet to investigate interactions between these factors and examine them alongside the metabolism of infant cortisol. Research has also not yet investigated variations in the metabolism of infant cortisol level over the first year of life and its impact upon weight gain and the development of obesogenic eating behaviours. Whilst the relationship between maternal sensitivity and infant cortisol is well established, no studies have yet assessed this relationship within the context of mother-infant feeding interactions. Given that eating behaviours have been shown to persist from childhood to adulthood (Ashcroft et al., 2008; Lien et al., 2001) and that obese children are more likely than their non-obese counterparts to become obese adults (Freedman et al., 1999), it is therefore of great importance to further understand the contributors to obesogenic eating behaviours and weight in infancy.

1.7 Aims and hypotheses

The overall aim of this thesis is to investigate the roles of breastfeeding, positive maternal mealtime interactions and cortisol metabolism on weight gain and the

development of eating behaviours during the first year of life, whilst controlling for covariates. In order to achieve this, the first aim of the thesis is to investigate the relationship between breastfeeding duration and infant weight and eating behaviours during the first year of life, whilst accounting for key confounding variables and overcoming issues of retrospective self-report encountered by previous research. It is hypothesised that infants breastfed for longer durations will show slower weight gain over the first postnatal year and demonstrate eating behaviours that are obesity-protective at 12-months.

The second set of aims refers to the investigation of cortisol metabolism. The objectives are to explore the development of infant cortisol metabolism and investigate the possible associations between maternal and infant cortisol metabolism, and cortisol metabolism and bodyweight, during the first year of life. It is hypothesised that there will be a positive relationship between maternal and infant cortisol metabolism and that there will be an association between infant cortisol metabolism and weight gain over the first year of life. For example, it is possible that infants with steroid ratios indicative of increased 11 β -HSD1 will be heavier than those with ratios indicative of lower 11 β -HSD1.

The thesis also aims to investigate associations between breastfeeding duration, positive maternal mealtime interactions, cortisol metabolism and eating behaviours. The thesis will then explore the key variables that may predict infant eating behaviours at 12-months and weight gain over the first year of life. It is hypothesised that infants breastfed for longer durations will have mothers who display more

positive interactions during mealtimes. These infants will have different patterns of cortisol metabolism and demonstrate healthier eating behaviours. Finally, it is hypothesised that breastfeeding duration, positive maternal mealtime behaviours and steroid ratios indicative of cortisol metabolism will all be significant predictors of infant weight gain and eating behaviours at 12-months. This study will not examine positive maternal interactions outside of the mealtime context. Figure 1.5 illustrates relationships found in published research and those hypothesised in this thesis.

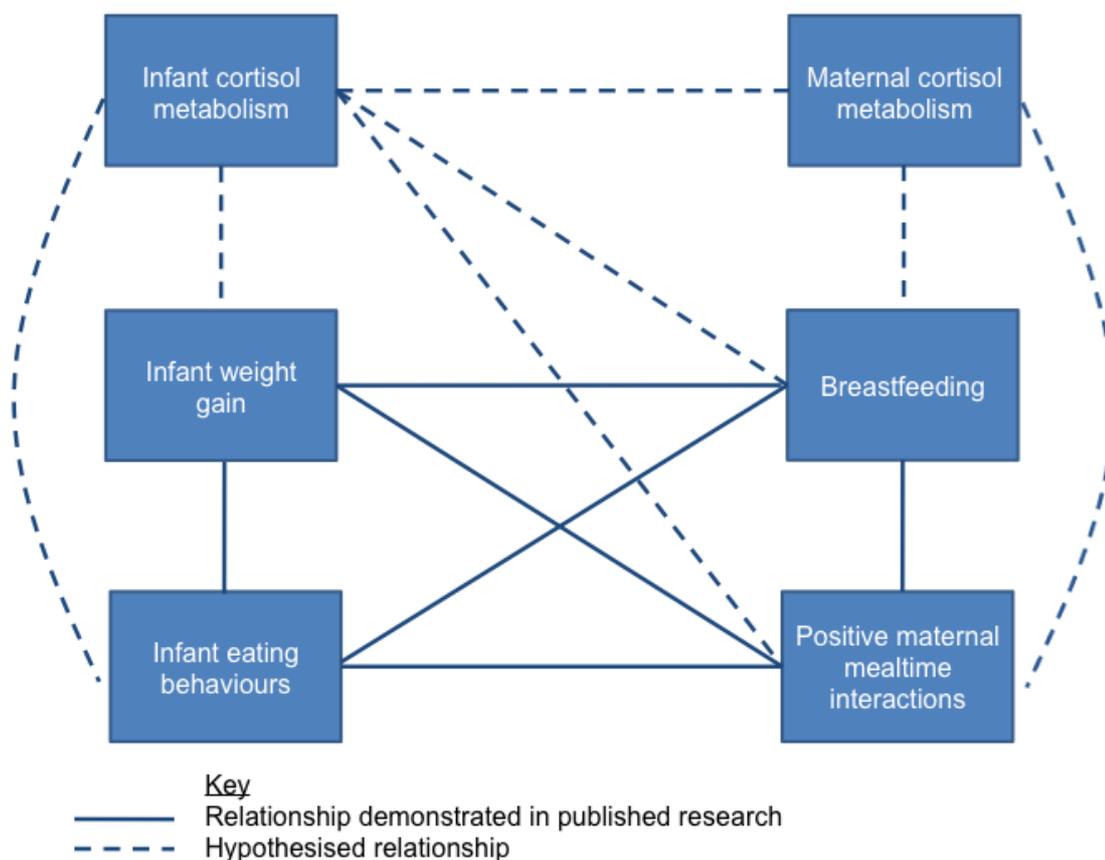


Figure 1.5

Model illustrating evidence-based and hypothesised relationships between infant and maternal cortisol metabolism and behaviour.

CHAPTER 2

METHODOLOGY

2.1 Ethics and governance

Ethical approval for this study was granted by Birmingham East, North and Solihull Research Ethics Committee, UK (reference 10/H1206/67); Research and Development clearance was granted by Birmingham Women's NHS Foundation Trust (reference 10/BWH/NO95).

2.2 Recruitment

Women were approached and given information about the study over a 7-month period between January and August 2011, on low risk maternity units of Birmingham Women's Hospital. During recruitment, midwives on low risk maternity units identified to the researcher, women who had no known complications during pregnancy and labour. After giving birth, midwives asked women if they were happy to speak to a researcher from the University of Birmingham. Upon the midwives' advice, women who agreed were then approached by the researcher. Potential participants were given a study information sheet (Appendix 2) on the maternity unit and consent was obtained for the researcher to telephone them 2- to 3-days later to assess willingness to participate in the study (Appendix 3). A second information sheet (Appendix 4) was also given to potential participants, which detailed the nappy collection procedure for the measurement of infant cortisol metabolism should they decide to take part in the

study. If upon telephone contact, potential participants stated that they would like to participate in the study, the researcher then visited them at home when their infant was approximately 1-week old. During this visit, any final questions were answered by the researcher and consent forms were signed by researcher and participant (Appendix 5). Consent forms requested maternal consent to: participate in the completion of questionnaires; to have their infants weighed and measured; to be observed feeding their child and to have this observation recorded; and donate wet nappies for the measurement of infant cortisol metabolism and urine samples from themselves for the measurement of maternal cortisol metabolism.

2.3 Participants

The researcher telephoned women 2-3 days after they had given consent to be contacted about the study. If they were still interested in participating, they were visited at home when their infant was approximately 1-week old. Infants born prematurely (prior to 36-weeks gestation) or small for gestational age were not eligible for this study as these factors may be associated with later growth and glucocorticoid metabolism (Casey, 2008; Clark et al., 1996). Individuals who could not read or write English were not included due to the requirement of completing questionnaires and the ability to communicate with the researcher; unfortunately this study did not have resources for translators or interpreters.

In total, 81 mother-infant dyads were recruited into the study and completed the 1-week home visit; please refer to Figure 2.1 for further information regarding the

number of mothers approached, contacted and visited at each point during the study. Data was not collected regarding why some mothers who had consented to being contacted, did not consent to the 1-week visit. However, anecdotal evidence revealed that mothers may have declined to participate due to: tiredness, having other young children to look after in addition to their newborn, their partner not wishing them to take part and work commitments.

Figure 2.1

Recruitment flowchart; the number of mothers approached, contacted and visited at each stage of the study.



2.4 Design

A longitudinal design was utilised for this study in order to combat issues that previous research has encountered. For example, issues regarding the retrospective report of breastfeeding duration were addressed by mothers reporting feeding information throughout the first postnatal year. Issues relating to the self-report of weight were overcome by the utilisation of regular home visits, during which the researcher adopted a standardised procedure and measured maternal and infant weight using electronic scales.

The longitudinal design of this study meant that mothers and infants could be followed from birth to 1-year of age. A design of this nature allows the collection and analysis of data in the immediate postnatal period, a time which is very important in the development of later weight gain and obesity (Cole; 2007; Lanigan & Singhal, 2009; Taveras et al., 2009). Mothers and infants were seen/contacted at 1-week, 1-month, 3-months, 6-months and 12-months postpartum. Mothers were observed feeding their infant at both of the 6- and 12-month home visits. Table 2.1 details the variables assessed at each time point of the study and the measures used to assess them.

2.5 Materials and procedure

After the completion of consent forms, the measures for the 1-week home visit were administered (see Table 2.1 for all measures and time points of the study).

Participants were seen/contacted at several time points postpartum. Home visits took place at 1-week, 1-month, 6-months and 12-months. At 3-months, participants were contacted via telephone and urine samples were collected from those who had agreed to provide them. At the end of the 12-month visit, participants were asked if they were happy to consent to being contacted regarding a later follow-up study (not featured in this thesis. Forms requested maternal consent to: being contacted; for their infants to be weighed and measured; and to provide another urine sample at 2-years-old (Appendix 6).

2.5.1 Questionnaires

Demographic and Additional Information (Appendix 7)

Mothers completed a demographic and additional information sheet during the 1-week visit. Mothers reported age, pre-pregnancy weight, ethnic background, household income, educational level achieved and infant date of birth. Each mother also reported how she initially planned to feed her baby.

Additional Information (Appendix 8)

Mothers completed an additional information sheet at the 1-, 3-, 6- and 12-month visits during the study. It requested information regarding medications currently being taken by the mother and present smoking status and weekly alcohol consumption.

Feeding Diary (Appendix 9)

Mothers were given feeding diaries at each time point of the study to complete for one week. This feeding diary aimed to support the information reported in the feeding information sheet. It assessed the duration and exclusivity of breast- and formula-feeding by detailing how many feeds the infant took per day and the source, quantity and duration of each feed. Unfortunately the data obtained from these diaries was unusable. Many mothers either did not complete them at all, or they completed them retrospectively during the home visits. Data from these diaries was therefore not included in the thesis.

Feeding Information (Appendix 10)

Mothers were asked at each time point, questions regarding feeding in order to establish whether infants were being breast- or formula-fed, and to determine the duration and exclusivity of feeding method. Questions asked the average quantity and duration of feeds the infant took per day around the time of the visit. At the later time points, questions also asked about the introduction of solid foods and the types of foods that infants were being fed. This information allowed the investigation of concurrent relationships between infant weight and breastfeeding duration throughout the first year of life, instead of examining early infant weight alongside the total breastfeeding duration over 12-months. The term 'concurrent breastfeeding duration' is referred to throughout the thesis and relates to the amount of time an infant has been breastfed up until the point in time being investigated. For example, a correlation investigating 6-month infant weight and concurrent breastfeeding duration

would be looking for a relationship between infant weight at 6-months and the amount of time an infant has been breastfed for, up until the age of 6-months.

Edinburgh Postnatal Depression Scale (EPDS; Cox, Holden & Sagovsky, 1987)

The EPDS (Appendix 11) was given to mothers at the 1-, 6- and 12-month visits to control for postnatal depression. It is a valuable and efficient way of identifying risk of postnatal depression (Cox et al., 1987). The EPDS is easy to administer consisting of 10 short statements. Responses are made using a tick box on a four-point Likert scale. For example, the item 'I have been anxious or worried for no good reason' has the following response choices, 'No, not at all', 'Hardly ever', 'Yes, sometimes' and 'Yes, very often'. Scores of 10 or greater are indicative of possible depression; scores above 13 are indicative of a depressive illness of varying severity. The scale indicates how the mother has felt during the previous week and takes approximately 5-minutes to complete. The EPDS is the most widely used screening questionnaire for postnatal depression (Boyd, Le & Somberg, 2005) and has been found to demonstrate moderate to good reliability properties across samples from a wide variety of countries and languages (Eberhard-Gran, Eskild, Tambs, Opjordsmone, Samuelson, 2001). The test-retest reliabilities are good to moderate. Regarding validity, a wide range of sensitivity and specificity values have been found but the EPDS does demonstrate good to moderate correlations with other depression measures (Eberhard-Gran et al., 2001). The Cronbach's alpha was .82 at 1-month, .83 at 6-months and .86 at 12-months.

Child Eating Behaviour Questionnaire (CEBQ; Wardle, Guthrie, Sanderson & Rapoport, 2001).

The CEBQ (Appendix 12) was given to measure maternal perception of infants' obesogenic and obesity protective eating behaviour at the 12-month visit. This measure was modified so that all questions to be asked were appropriate for mothers of 12-month-old infants. Therefore, the emotional over- and under-eating subscales were not given as they are not appropriate for infants aged 12-months-old. The original CEBQ consists of 35-items and the current modified version consists of 23-items. Responses are made using tick boxes on a five-point rating scale ranging from 'never' to 'always'. The modified version was piloted on 59 mothers of infants with a mean age of 7.5-months. Overall reliability was shown to be good to moderate (.62).

Items of the CEBQ were derived from research into the behavioural causes of obesity and from parent interviews. Eight subscales (and example items) include: (1) satiety responsiveness, 'My infant gets full up easily'; (2) enjoyment of food, 'My infant loves food'; (3) food responsiveness, 'If allowed, my infant would eat too much'; (4) slowness in eating, 'My infant eats slowly'; (5) food fussiness, 'My infant is difficult to please with meals'; and (6) desire to drink, 'If given the chance, my infant would always be having a drink'. The CEBQ has been shown to demonstrate high internal validity and test-retest reliability (Wardle et al., 2001) and has been used to investigate whether differences in feeding practices are related to differences in siblings' eating behaviours (Farrow, Galloway & Fraser, 2009) and also the contribution of genes and environment to appetite (Carnell, Haworth, Plomin & Wardle, 2008). The Cronbach's alphas were .83 for enjoyment of food and satiety

responsiveness, .74 for slowness in eating, .84 for food fussiness, .85 for responsiveness and .88 for desire to drink.

Table 2.1

Variables assessed and measures used at each time point of the study.

Time Point	Variable	Measures	Variable Type
1-week	Infant cortisol metabolism	24-hour sample of wet nappies	Predictor
	Maternal cortisol metabolism	Single urine sample	Predictor
	Infant weight and length		Outcome
	Maternal weight and height		Covariate
	Background information, medication, smoking and alcohol consumption	Demographic and additional information sheet	Covariate
	Feeding information	Feeding information sheet and diary	Predictor
1-month	Infant cortisol metabolism	24-hour sample of wet nappies	Predictor
	Maternal cortisol metabolism	Single urine sample	Predictor
	Infant weight and length		Outcome
	Maternal weight		Covariate
	Maternal current medication, smoking and alcohol consumption	Additional information sheet	Covariate
	Feeding information	Feeding information sheet and diary	Predictor
	Postnatal depression	EPDS	Covariate
3-months	Infant cortisol metabolism	24-hour sample of wet nappies	Predictor
	Maternal cortisol metabolism	24-hour urine sample	Predictor
	Maternal current medication, smoking and alcohol consumption	Additional information sheet	Covariate
	Feeding information	Feeding information sheet and diary	Predictor

Time Point	Variable	Measures	Variable Type
6-months	Infant cortisol metabolism	24-hour sample of wet nappies	Predictor
	Maternal cortisol metabolism	Single urine sample	Predictor
	Infant weight and length		Outcome
	Maternal weight		Covariate
	Maternal current medication, smoking and alcohol consumption	Additional information sheet	Covariate
	Postnatal depression	EPDS	Covariate
	Feeding information	Feeding information sheet and diary	Predictor
	Maternal and infant feeding interaction	Observation, FIS and rating of meal representativeness	Predictor
12-months	Infant cortisol metabolism	24-hour sample of wet nappies	Predictor
	Maternal cortisol metabolism	Single urine sample	Predictor
	Infant weight and length		Outcome
	Maternal weight		Covariate
	Maternal current medication, smoking and alcohol consumption	Additional information sheet	Covariate
	Postnatal depression	EPDS	Covariate
	Feeding information	Feeding information sheet and diary	Predictor
	Infant eating behaviours	CEBQ	Outcome
	Maternal and infant feeding interaction	Observation, FIS and rating of meal representativeness	Predictor

2.5.2 Observation

Feeding Interaction Scale (FIS; Wolke, Summer, McDermott & Skuse, 1987).

The FIS was used to code the observations of the mother-infant feeding interaction at 6- and 12-months. Three subscales were used to assess maternal behaviours including frequency of verbal involvement, appropriateness and sensitivity of maternal behaviour (refer to Table 2.2 for further detail on the subscales of the FIS). Frequency of maternal vocalisations and appropriateness during a meal were analysed in addition to maternal sensitivity to allow the investigation of several positive feeding behaviours. The FIS also counts the number of offers by the mother, acceptances, self-feeds and spoons of food consumed by the infant. The FIS has clinical validity and has been used to assess maternal-infant feeding interactions and diagnose feeding problems (Farrow & Blissett, 2005; Lingberg, Bohlin, Hagekull & Palmerus, 1996; Skuse, Wolke & Reilly, 1992).

Table 2.2

Subscales and behaviours utilised from the FIS (Wolke et al., 1987).

Subscale	Behaviour	Scoring
Maternal verbal involvement	Proportion of session mother is talking to infant including initiating conversation and spontaneous comments	1 (never talks to infant) to 9 (very much)
Appropriateness of maternal mealtime behaviour	Feeding is appropriate if it is pleasurable for mother and infant.	1 (very inappropriate) to 5 (very appropriate)
Maternal sensitivity	Infant in sensible position including freedom of arm movement and eye contact with mother, close proximity to mother, feedback on infant's behaviour, variation of stimulation	1 (highly insensitive) to 9 (highly sensitive)

2.5.3 Measurement and analysis of cortisol metabolism

The analysis of cortisol metabolism requires the use of methods that are powerful, reliable and free from interference. This is particularly true when examining the unconjugated free steroids, as they are present in extremely low amounts (Cuzzola, Petri, Mazzini & Salvadori, 2009). Various procedures have been utilised for the clinical assay of glucocorticoids; in the past, immunoassay-based methods were the method of choice for the analysis of cortisol (Nozaki, 2001). Immunoassays however, are prone to interference from other sterols, including cortisone, and are generally unsuitable for the analysis of glucocorticoid metabolites. Pujos, Flament-Waton, Paisse and Grenier-Loustalot (2005) examined methods for analysis of endogenous and synthetic corticosteroids present in urine using enzyme-linked immunosorbant assay (ELISA), gas chromatography coupled with mass spectrometry (GC/MS), and liquid chromatography coupled with mass spectrometry (LC/MS). It was concluded that for a total assay when screening for endogenous corticosteroids (such as cortisol and its metabolites), the ELISA technique cannot be used due to the possibility of false-positives occurring in the analysis. Pujos et al., (2005) state that it is necessary to use chromatographic techniques for specific analysis and that GC/MS is more sensitive and enables better separation of compounds than LC/MS. Cuzzola et al., (2009) evaluated GC/MS and LC/MS for the measurement of free cortisol, cortisone and their unconjugated tetrahydrometabolites in the urine of human adults. GC/MS was found to be the more convenient method in terms of sensitivity and separation efficiency. It was also stated that GC/MS allowed for corticosteroids to be analysed without derivatization.

Investigating cortisol metabolism in the first year of life requires the utilisation of a method that is powerful, reliable and sensitive enough to detect steroid metabolites present in extremely small amounts. It is important for ethical reasons, that the method be as non-invasive and comfortable as possible for the infant. It is also important for practical reasons that the method is uncomplicated, as this will help mothers to successfully and reliably collect 24-hour samples of wet nappies from their infant. The method of steroid extraction from nappies is novel and meets the aforementioned requirements.

Prior development of the method has established that disposable nappies consist of cellulose fluff containing 'supersorb' granules (polyacrylate resin) and that there are no material differences between the major brands, including Pampers (Proctor and Gamble) and Huggies (Kimberly-Clark). Samples of the resin did not selectively retain steroids. The saline concentration was established at 4% to minimise gel swelling. Experiments involving dripping urine onto new nappies established that steroid recovery was close to 100%.

Mothers were asked to collect 24-hour samples of wet nappies at each time point during the study; they were reminded to do so 24- to 32-hours prior to each visit. Full instructions were given to the families (Appendix 4). Mothers were also requested to provide a single urine sample at the 1-week, 1-, 6- and 12-month visits and if willing, a full 24-hour sample at the 3-month collection. The purpose of the 24-hour collection was to attain a fuller picture of maternal cortisol metabolism for reference. Cortisol and cortisone excretion varies over a 24-hour period; therefore ratios in a single

sample could be different to those in the 24-hour sample, depending on the time that it was collected. Obtaining a 24-hour urine sample evens out the well-known circadian fluctuation in hormone levels and enables the investigation of the proportion of active and inactive glucocorticoid within a 24-hour period. Infant nappy and maternal urine samples were stored in freezers at the Institute of Biomedical Research at the University of Birmingham until they were ready for analysis.

Before the extraction process, nappy collections were defrosted for 24-hours in the cold room. The contents of the wet nappies were extracted in the lab using detailed instructions (Appendix 13); records were kept throughout each extraction (Appendix 14). Once nappy extractions were completed, they were then analysed by a trained and reliable lab technician using gas chromatography/mass spectrometry (GC/MS; see Appendix 15 for instructions that were followed). GC/MS is a method used for the analysis of metabolites of steroid hormones and a scanned run will detail every steroid excreted; it is superior to immunoassays regarding specificity, especially with low concentrations of the metabolites being assessed (Krone et al. 2010).

As described in Chapter 1, the enzymes responsible for the interconversion of cortisol and cortisone are 11β HSD2, 11β HSD1 and 5α -reductase. From the GC/MS analysis, three ratios were calculated that reflected activity of these enzymes:

- 1.) The ratio of urinary cortisol to cortisone (F/E), reflects 11β -HSD2 activity (Palermo, Shackleton, Mantero & Stewart, 1996).

- 2). The ratio of tetrahydro-metabolites of cortisol to those of cortisone (THF + 5 α THF/THE) provides a reflection of 11 β -HSD1 activity.
- 3). The activities of 5 α -reductase were inferred from measuring the ratio of 5 α THF/THF.

Figure 2.2 illustrates that higher F/E indicates lower activity of 11 β -HSD2, higher THF + 5 α THF/THE indicates greater activity of 11 β -HSD1 and higher 5 α THF/THF indicates more activity of 5 α -reductase.

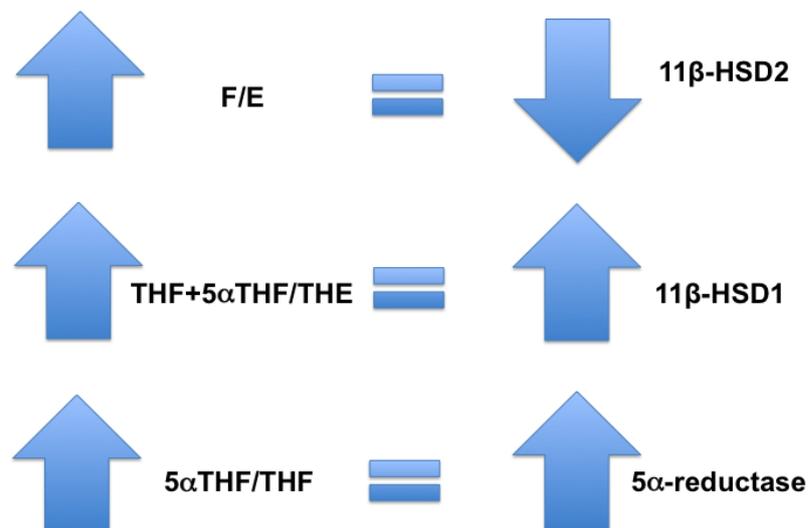


Figure 2.2

Illustration describing the meaning of higher levels of steroid ratios in terms of enzyme activity.

2.5.4 Anthropometric measures

Infants were weighed naked with electronic scales by the researcher at 1-week, 1-, 6- and 12-months. Weight was converted to a standard deviation score (SDS) which adjusts measurements for age and gender (Child Growth Foundation, 1996). Weight

gain SDS was also calculated, which measures the change in weight SDS from 1- to 6-, 1- to 12- and 6- to 12-months. Weight gain SDS therefore compares an infant's current weight with the weight they were predicted to be based on their previous weight and is a valid measure of weight gain of British infants from the age of 4-weeks (Cole, 1995). As with any standard deviation, a difference of 1 SDS of weight is "large", a difference of 2 SDS of weight is "very large" and a difference of 3 SDS of weight is "extremely large". Furthermore, a person who is 1 standard deviation above the mean is at the 84th percentile; a person who is 2 standard deviations above the mean is at the 98th percentile.

Infant length was measured at each home visit to the nearest 0.5cm using a Seca 210 mobile measuring mat. Mothers were weighed wearing light indoor clothing without shoes using electronic scales at 1-week, 1-, 6- and 12-months. Maternal height was measured at 1-week postpartum using a stadiometer.

2.6 Sample characteristics

Of the 81 mother-infant dyads that were visited at 1-week, 72.84% of these agreed to provide urine samples from themselves and their infant at each time point for the analysis of cortisol metabolism. By the end of the 12-month follow-up home visit, only 15% of mothers had withdrawn from the study. Reasons for not continuing with the research included disinterest with the study, lack of time to complete visits, changing telephone number and not responding to letters and moving house and not forwarding the new address.

Women who withdrew did not significantly differ from those who remained in the study in terms of: household income, how many other children they had, alcohol consumption (during pregnancy and at 1-week, 1-, 3-, 6-months postpartum), signs of postnatal depression at 1- and 6-months (a score of 10 or greater on the EPDS was accepted as indicative of possible depression) or infant birth weight (see Appendix 16). However, women who withdrew did significantly differ from those who remained in the study in their educational level, age and how many cigarettes they smoked at 1-week (but not during pregnancy, 1-, 3- or 6-months). Those who remained in the study had reached a higher level of education than those who withdrew ($t = -2.77$, $df = 79$, $p < .01$). Those who remained in the study were older than those who withdrew ($t = -2.30$, $df = 79$, $p < .05$). In addition to this, those who remained in the study reported smoking significantly less at 1-week postpartum (but not during pregnancy or at 1-, 3- and 6-months) than those who withdrew ($t = 2.25$, $df = 11.48$, $p < .05$). Despite these differences, the overall drop-out rate for this study was still very low (14.8%).

The mean age of women at the 1-week visit was 29.42 years (SD 5.87) and 45.7% had at least an undergraduate degree; 11.1% had left school before the age of 16 years. The sample was predominantly White; 58% were White British and 12.3% were Asian Pakistani. The remaining 68% included: White Irish, White Other, Asian Indian, Black Caribbean, Black African, Black Other and Mixed Heritage. Infants included 46 males and 35 females with a mean birth weight of 3.52kg (SD 0.39).

Using a cut-off of 10 or greater on the EPDS is likely to identify almost all cases of postnatal depression, whilst generating very few false negatives. This cut-off is particularly useful for research projects in which the EPDS is the only measure of depression to be used (Cox et al., 1987). Up to 15% of women are affected by postnatal depression after giving birth (Royal College of Psychiatrists; RCPSYCH, 2012). The rates of postnatal depression in the current sample are comparable to those seen in the general population. Table 2.3 details the number of women showing signs of postnatal depression at the 1-, 6- and 12-month home visits.

Table 2.3

Number of women showing signs of postnatal depression (as measured by the EPDS) at the 1-, 6- and 12-month home visits.

	10 or greater		Above 13	
	<i>Possible depression</i>		<i>Likely to be suffering from depressive illness of varying severity</i>	
	N	%	N	%
1-month	14	18.18%	5	6.49%
6-months	13	17.81%	2	2.74%
12-months	11	15.94%	4	5.80%

At the 6-month home visit, 79.45% of the mother-infant dyads were observed during a mealtime. Mothers who were observed during a mealtime were not significantly different from those who were not observed according to their age, household income, education, how many other children they had, the number of cigarettes smoked, signs of postnatal depression or infant SDS weight (see Appendix 17). Women who were observed did significantly differ in the units of alcohol they were

consuming; those who were observed with their infant during a mealtime reported consuming an average of 1 to 13 units of alcohol per week whereas those more those who were not observed reported consuming an average of 0 units of alcohol per week ($t = 2.16$, $df = 71$, $p < .05$).

At the 12-month home visit, 79% of the mother-infant dyads were observed during a mealtime. Mothers who were observed during a mealtime were not significantly different from those who were not observed according to their age, household income, how many other children they had, the number of cigarettes smoked and alcohol units consumed per week, signs of postnatal depression or infant SDS weight (see Appendix 17). Women who were observed did significantly differ from those who were not observed in their educational level; those who were observed with their infant during a mealtime reached a higher level of education than those who were not observed ($t = 2.27$, $df = 67$, $p < .05$).

2.7 Data analysis

Analyses throughout the thesis all refer to the same longitudinal study. Due to the complexity of the data set and the different angles that were taken in the examination of the data, the analysis has been split into several chapters. Chapter 3 investigated the relationships between breastfeeding duration and infant weight and eating behaviours; Chapter 4 took a more exploratory stance and provided normative data on infant cortisol metabolism and investigated its association with bodyweight during the first year of life; Chapter 5 examined potential associations between

breastfeeding duration, positive maternal interactions during feeding and infant cortisol metabolism at 6- and 12-months-of-age; and Chapter 6 has attempted to bring together the important aspects of analyses from preceding chapters with the aim of predicting eating behaviours and weight gain during infancy.

Outcome variables were infant weight gain across the first year and eating behaviours as rated by the mother at 12-months and observed by the researcher at 6- and 12-months. Predictor variables included breastfeeding duration (in weeks), observed maternal positive interactions during feeding and infant cortisol metabolism (obtained from urine samples). Covariates included household income, maternal age, education, BMI and quantity of cigarettes smoked during pregnancy, postnatal depression, infant gender, birth weight SDS and age at which they were introduced to solid food.

The focus of the proposed study was to investigate predictors of infant weight gain and eating behaviours. In addition to testing some specific hypotheses, data were also examined in an exploratory manner where appropriate. Kolmogorov-Smirnov tests and histograms indicated that breastfeeding duration, demographic factors, postnatal depression and most steroid ratios were not normally distributed. Therefore, in order to assess potential covariates that needed to be controlled in subsequent analyses, two-tailed non-parametric Spearman's rho correlations were used to test whether a linear relationship existed between demographic variables and feeding practices, cortisol metabolism and infant weight gain. Those significantly associated with variables of interest were then controlled for in further analyses.

Parametric tests were used in Chapters 3, 4, 5 and 6 as Kolmogorov-Smirnov tests and histograms indicated that most observed maternal interactions and the outcome variables, infant weight, weight gain and eating behaviours, were normally distributed. Furthermore, it was essential to investigate potential associations between variables whilst controlling for covariates.

Within Chapters 3, 4, 5 and 6, partial correlations have been used to assess relationships between breastfeeding duration, positive maternal feeding interactions and infant weight gain, eating behaviours and cortisol metabolism, whilst controlling for covariates. Results of these partial correlations have been used to select significant variables to use in regression analyses in Chapter 6.

In Chapter 3, one-tailed partial correlations were used to assess relationships between: (1) breastfeeding duration and infant weight and weight gain throughout infancy; (2) breastfeeding duration and infant eating behaviours at 12-months; and (3) infant weight SDS and eating behaviours at 12-months.

Chapter 4 began by using mixed effects models in order to test whether maternal steroid ratios change over time and whether infant ratios change over time and by gender. Such data sets often present with correlated errors that violate the inference assumptions of standard ANOVA and regression models. Therefore, these data required analysis using a mixed fixed and a random factor linear model, which use the random effects to model those sources of variation attributable to the design of the study. The mixed methods analyses in Chapter 4 were carried out in conjunction

with Dr Christopher Jones, a statistics advisor in the School of Psychology at the University of Birmingham. Chapter 4 also included one-tailed partial correlations to investigate relationships between maternal and infant steroid ratios indicative of cortisol metabolism and two-tailed partial correlations to examine potential relationships between infant weight, weight gain and steroid ratios throughout the first year of life.

Chapter 5 used one-tailed partial correlations to investigate relationships between breastfeeding duration and observed positive maternal interactions during solid feeding at 6- and 12-months. More exploratory two-tailed partial correlations were then used to investigate potential relationships between: (1) breastfeeding duration and infant steroid ratios indicative of cortisol metabolism; and (2) positive maternal feeding interactions and infant steroid ratios, whilst controlling for covariates.

Chapter 6 used one-tailed partial correlations to investigate relationships between positive maternal mealtime interactions and infant food acceptances and eating behaviours, as measured by the CEBQ. Two-tailed partial correlations were then conducted to investigate the potential relationships between infant steroid ratios and infant food acceptances and eating behaviours. Lastly, regression analyses were performed to predict infant weight gain and eating behaviours. To decide which key variables to include in the analyses, it was decided that eating behaviours significantly related to variables of interest in Chapters 3 and 6 would be predicted. As these analyses were exploratory in nature and it was unclear which of the variables would be the primary predictor, stepwise regressions were used whereby

the order in which predictors are entered into the model is based on mathematical criterion.

CHAPTER 3

**BREASTFEEDING DURATION, WEIGHT GAIN AND EATING BEHAVIOURS AT
12-MONTHS**

Abstract

Background: Results of research investigating relationships between breastfeeding and infant weight gain are not consistent. Few studies account for most significant covariates and many studies have substantial methodological problems such as retrospective self-report of information. The current study aimed to investigate the relationship between breastfeeding duration and infant weight and eating behaviours during the first year of life, whilst accounting for key confounding variables and overcoming issues of retrospective self-report.

Method: Eighty-one mothers and infants were recruited from low-risk maternity units in Birmingham and visited at home at 1-week, 1-, 6- and 12-months postpartum. Mothers and infants were weighed and measured and feeding information was recorded at each visit. Infant weight was converted to a standard deviation score (SDS), which accounted for age and gender. Mothers reported infant eating behaviours at 12-months using an age-appropriate version of the CEBQ.

Results: Partial correlations (controlling for: maternal age, education, BMI, number of cigarettes smoked during pregnancy, household income, infant birth weight SDS and age introduced to solids) revealed that duration of breastfeeding was significantly negatively associated with weight SDS at 6- and 12-months, but not 1-week or 1-month, weight gain SDS from 1- to 6- and 1- to 12-months, and a slower rate of infant

eating at 12-months. Heavier infants at 12-months were reported by their mothers to enjoy food more, eat more quickly and be less satiety responsive than lighter infants.

Discussion: Breastfeeding duration is associated with slower weight gain and lower weight at 6- and 12-months. Results support a dose-dependent effect of breastfeeding on weight gain. This effect remained true after controlling for significant covariates. In the UK, breastfeeding rates are currently lower than that desired; strategies to promote breastfeeding are important and are likely to result in significant benefits for health.

3.1 Introduction

Eating behaviours persist from childhood into adulthood (Ashcroft et al., 2008; Lien et al., 2001) and obese children are more likely to become obese adults than non-obese children (Freedman et al., 1999). It is therefore of great importance to further develop our understanding of the contributors to excessive weight and its associated eating behaviours in infancy so that more effective preventions and interventions can be developed.

Interventions that are targeted at helping obese children lose weight are often expensive to health services and do not have good success rates in the long-term. For example, an intervention (utilising motivational interviewing) designed to reduce television watching and consumption of fast food and sugary drinks was found to result in less television viewing but did not reduce child BMI (Taveras et al., 2011). In addition to this, research has demonstrated that children often struggle with weight

management interventions when their parents also have a weight problem (Fassihi, McElhone, Feltbower & Rudolf, 2012). It is therefore vital to identify and better understand preventative measures that programme healthier patterns of weight gain and eating behaviours in order to reduce paediatric obesity.

For the last 30 years, research has been conducted into whether breastfeeding protects against rapid weight gain, overweight and obesity later in life. Studies have provided evidence for the protective dose-dependent effect of breastfeeding on overweight and obesity in childhood and adolescence (Arenz et al., 2004; Hörnell et al., 2013; Kramer, 1981; McCrory & Layte, 2012; Owen et al., 2005). Research has also demonstrated that breastfed infants gain less weight during the critical neonatal period than formula-fed infants (Heinig et al., 1993). Infants who gain less weight during this period are less at risk of becoming obese later in life (Stettler et al., 2002).

Various mechanisms have been suggested for breastfeeding's protective effect; these include: the caloric and protein content of breast milk versus formula milk (Alexy et al., 1999), breastfed infants may be able to better attend to internal signals of hunger and satiety (Birch & Fisher, 1998), facilitation of maternal sensitivity (Brown & Lee, 2013; DiSantis et al., 2013), maternal experience of allowing the infant to control its own intake (Birch & Fisher, 1998), and the biologically active ingredients within breast milk (Miralles et al., 2006).

In addition to breastfeeding's effect on weight, it is also related to the development of healthy eating behaviours, such as increased consumption of fruits and vegetables

(Kudlová & Schneidrová, 2012; Menella et al., 2001). Breastfed infants are also found to be more satiety responsive (Brown & Lee, 2012) and greater satiety responsiveness is related to a lower risk of being overweight in childhood (Webber, Hill, Saxton, van Jaarsveld & Wardle, 2009). This increased satiety responsiveness may arise because breast milk changes in energy content, both throughout the day and within each feed (Jenness, 1979; Nommsen et al., 1991). Breastfed infants may therefore learn to adapt their intake according to the ever-changing fat content of the milk.

Findings however, are not consistent. Some studies claim the effect of breastfeeding on childhood obesity is 'trivially small' (Jiang & Foster, 2013) and others have found no effect at all (Davis et al., 2007; Martin et al., 2013; Novaes, Lamounier, Colosimo, Franceschini & Priore, 2011; Oddy et al., 2004). It is important to highlight here that the reason for null findings may be due to a lack of control of covariates and a range of other methodological issues. There is a wide variation between studies with regards to the covariates accounted for; examples include, infant birth weight, gender and gestational age, maternal age, BMI, smoking status during pregnancy (and postnatally), maternal diabetes, educational level and household income (see Chapter 1 'Demographic and Psychological Factors' for a more extensive review). Very few studies in the published literature account for all of these variables. Methodological problems in this area involve (but are not exclusive to) retrospective data collection, inconsistent definitions of breastfeeding (including exclusivity and duration), small sample sizes or the same data from larger samples being used several times, and maternal self-report of height, weight and breastfeeding history.

Despite the inconsistencies within the research, breastfeeding's protective effect is highlighted by the results of Harder et al.'s (2005) meta-analysis, which found that a longer duration of breastfeeding was associated with a reduced risk of becoming overweight. Furthermore, a multivariate analysis of 7,798 children in Ireland, which controlled for a variety of covariates including socio-demographic factors, child birth weight, gender and physical activity and parental BMI, demonstrated that children who had been breastfed for 13- to 25-weeks had a 38% reduction in the risk of being obese at 9-years-of-age (McCrary & Layte, 2012). Furthermore, breastfeeding for at least 26-weeks was associated with a 51% reduction in obesity risk at 9-years-of-age. These results also supported the dose-dependent effect of breastfeeding for durations greater than 4-weeks (McCrary & Layte, 2012).

The mechanisms by which breastfeeding protects infants against excessive weight gain therefore require further research. In order to do this, it would be useful to investigate the relationships between breastfeeding duration and infant weight gain and eating behaviours together in one study. Currently, there is no longitudinal study published that investigates all of these factors together over the first postnatal year. By doing this, we will learn more about the links between breastfeeding, and the patterns of weight gain and eating behaviours that are obesity-protective.

It is essential that any study intending to investigate breastfeeding's effect on infant weight gain and eating behaviours takes into account the aforementioned methodological shortcomings. The current study aimed to achieve this by recruiting a sample of women who had no complications, such as gestational diabetes, during

pregnancy. Mother-infant dyads were visited at home at several time points over the first postnatal year and feeding information was recorded, rather than asking for such information retrospectively. Also, the researcher measured height and weight of mothers and infants rather than asking participants to self-report these details.

3.1.1 Aims and hypotheses

The aim of this study was to investigate the relationship between breastfeeding duration and infant weight and eating behaviours during the first year of life. It is hypothesised that infants breastfed for longer durations will: (1) show slower weight gain between 1- and 6- and 1- and 12-months; (2) weigh less at 12-months than infants breastfed for shorter durations; and (3) demonstrate eating behaviours that are obesity-protective (as perceived by their mothers) at 12-months. It is also hypothesised that infants who are heavier at 12-months will be reported as having more obesogenic eating behaviours by their mothers.

3.2 Methods

3.2.1 Participants

The sample consisted of 81 full-term infants (45 males, 36 females; mean birth-weight 3.52 kg [SD 0.39]), and their mothers (mean age 29.42 years [SD 5.87]) who were recruited from low-risk maternity units of the Birmingham Women's Hospital (see Chapter 2 for more information on recruitment and ethical approval). Infants born prematurely (prior to 36-weeks gestation) or small for gestational age were not

eligible for this study as these factors may be associated with weight gain during the first 12-months of life. Mothers who could not read or write English were not included due to the requirement of completing questionnaires and the ability to communicate with the researcher.

3.2.2 Materials and procedure

Mothers were given information sheets on the maternity unit (Appendix 2 & 4) and consent was obtained for the researcher to telephone them to assess willingness to participate in the study (Appendix 3). Mothers happy to participate in the study were visited at home at approximately 1-week postpartum where consent forms were signed by the researcher and participant (Appendix 5).

Mothers and infants were visited at home at 1-week, 1-month, 6-months and 12-months postpartum. Mothers and infants were weighed and measured by the researcher at each visit. Mothers reported feeding information at each visit; this included details regarding breast- and formula-feeding exclusivity and duration and when solids were first introduced. At each visit mothers also reported their smoking and alcohol consumption and any medications they were taking. Demographics were reported at the 1-week visit. Mothers completed questionnaires assessing symptoms of postnatal depression at 1-, 6- and 12-months and their infant's eating behaviours at 12-months (see Table 2.1 in Chapter 2 for measures given at each home visit).

Demographic and Additional Information (Appendix 7)

Mothers completed a demographic and additional information sheet during the first week of recruitment to the study. It requested age, pre-pregnancy weight, ethnic background, household income, educational level achieved and infant date of birth. It also asked how the mother intended to feed her baby.

Additional Information (Appendix 8)

Mothers completed an additional information sheet, which requested information regarding medications currently being taken by the mother and present smoking and alcohol consumption.

Feeding Information (Appendix 10)

At each visit, mothers reported whether infants were being breast or formula-fed, and the duration and exclusivity of feeding method. At the later time points, questions were asked about introduction of solid foods and the types of foods infants were fed. This information allowed the investigation of coexisting relationships between infant weight and breastfeeding duration, rather than examining early infant weight alongside the total breastfeeding duration over the 12-months.

Edinburgh Postnatal Depression Scale (EPDS; Cox et al., 1987)

The EPDS was given to mothers at the 1-, 6- and 12-month visits to establish whether depression needed to be controlled for in the analyses. As explained in Chapter 2, it is a valuable and efficient way of identifying risk of postnatal depression (Cox et al., 1987). The EPDS consists of 10 short statements, each of which has four responses to choose from. It is therefore easy to administer and takes less than 5-minutes to complete. The scale indicates how the mother has felt during the previous week. Mothers who score 10 or greater are identified as showing symptoms indicative of possible depression.

Child Eating Behaviour Questionnaire (CEBQ; Wardle et al., 2001)

A modified age-appropriate version of the CEBQ (Appendix 12) was given at the 12-month visit to assess maternal perception of infants' obesogenic and obesity protective eating behaviours. As described in more detail in Chapter 2, it is a reliable and valid 35-item parent-rated questionnaire measuring eating styles of children using a five-point rating scale. Eight subscales include: (1) satiety responsiveness; (2) enjoyment of food; (3) food responsiveness; (4) slowness in eating; (5) food fussiness; (6) emotional over-eating; (7) emotional under-eating; (8) desire to drink. Subscales measuring emotional over- and under-eating were deemed not appropriate for infants 12-months-old and so were not given in this study.

Anthropometric Measures

As described in Chapter 2, infants were weighed naked with electronic scales by the researcher and weight was then converted to weight SDS. Infant length was measured to the nearest 0.5cm using a Seca 210 mobile measuring mat. Mothers were weighed wearing light indoor clothing without shoes using electronic scales and maternal height was measured at 1-week postpartum using a stadiometer.

3.2.3 Data analysis

Kolmogorov-Smirnov tests and histograms indicated that breastfeeding duration and demographic factors and postnatal depression were not normally distributed. Two-tailed non-parametric Spearman's rho correlations were therefore used to assess whether these variables were associated with breastfeeding duration.

For the main analyses, parametric tests were used as Kolmogorov-Smirnov tests and histograms indicated infant eating behaviours, weight and weight gain were normally distributed. In addition, it was an essential aspect of the study to investigate the relationships between breastfeeding and weight whilst controlling for confounding variables. One-tailed partial correlations (controlling for: household income category, maternal age, education, BMI and quantity of cigarettes smoked during pregnancy, infant birth weight SDS and age at which introduced to solids) were therefore used to assess the relationship between: (1) breastfeeding duration and infant weight SDS at 1-week, 1-, 6- and 12-months and weight gain SDS from 1- to 6- and 1- to 12-

months; (2) breastfeeding duration and infant eating behaviours at 12-months; and (3) infant weight SDS and eating behaviours at 12-months.

3.3 Results

3.3.1 Descriptive statistics

Eighty-one mother-infant dyads were initially recruited into the study; at the 12-month follow-up visit 12 had withdrawn, resulting in a dropout rate of 14.8%. Table 3.1 shows the number of mother-infant dyads seen at each home visit, the mean age of infants (in weeks) at each home visit and the percentage of infants being breastfed (includes exclusive breastfeeding and infants receiving both breast milk and formula). Of the 73% breastfeeding at 1-week, 75% of these mothers were exclusively breastfeeding. Of the 65% breastfeeding at 1-month, 76% of these were exclusively breastfeeding. Of the 52% breastfeeding at 6-months and 32% at 12-months, 71% and 64% had not introduced formula or cow's milk respectively. There was no difference in breastfeeding duration between male ($M = 24.34$, $SE = 3.58$) and female ($M = 30.58$, $SE = 4.03$) infants $t(67) = -1.16$, $p = .25$.

The mean age infants were introduced to solid food was 20.41 weeks (SD 3.39). There was no difference between male ($M = 20.20$, $SE = 0.60$) and female ($M = 20.65$, $SE = 0.51$) infants in the age at which they were introduced to solid food $t(71) = -.57$, $p = .57$.

Table 3.1

Number of participants visited at each point, mean age of infants (weeks) at each visit and percentage of infants receiving any breast milk at each visit.

Visit	N	Mean age (weeks)	Std. Deviation	Any breastfeeding
1-week	81	1.32	0.36	72.84%
1-month	77	4.77	0.62	64.94%
6-months	73	26.67	0.99	52.05%
12-months	69	52.83	1.73	31.88%

As demonstrated in Table 3.2 there were no male infants below the 2nd and there were no female infants below the 9th centile for weight at 1-, 6- or 12-months. These centiles were plotted using the UK-WHO growth charts (Appendix 1). The growth charts state that 99 out of every 100 infants who are growing optimally will lie between the 0.4th and 99.6th centile. The charts also state that there is not a cut-off for abnormal growth, but only 4 in every 1,000 infants growing optimally will be below 0.4th centile; these infants should be assessed to exclude any health problems. Therefore it can be stated that there were no significantly underweight infants in the current sample.

Table 3.2

Weight and centile range of male and female infants at each home visit according to the UK-WHO growth charts (Appendix 1).

Visit	N	Weight range (kg)		Centile range	
		Males	Females	Males	Females
1-week	81	2.72 – 4.88	2.81 – 4.37	n/a	n/a
1-month	77	3.43 – 6.00	3.74 – 5.39	2 nd – 98 th	9 th – 91 st
6-months	73	6.46 – 10.50	6.59 – 9.38	2 nd – 98 th	25 th – 98 th
12-months	69	8.00 – 12.81	7.71 – 11.82	2 nd – 99.6 th	9 th – 98 th

3.3.2 Covariates

A score of 10 or above was used to identify the participants showing signs of possible depression (Cox et al., 1987). At 1-month, 14 women (18.18%) were showing signs of possible postnatal depression, at 6-months 13 women (17.81%) were showing signs of possible postnatal depression and at 12-months 11 women (15.94%) were showing signs of possible postnatal depression. EPDS score was not significantly associated with breastfeeding duration at: 1-month $r=.21$, $p>.05$; 6-months $r=.06$, $p>.05$; or 12-months $r=.16$, $p>0.05$. Therefore, postnatal depression was not controlled for in any further analyses.

Two-tailed Spearman's rho correlations revealed that maternal age and educational level were significantly associated with breastfeeding duration at each time point of the study (see Table 3.3). There were positive associations between breastfeeding duration and household income at 1-week, 1- and 6-months; positive associations between breastfeeding and age infants were introduced to solid food at 6- and 12-

months; and negative associations between breastfeeding and cigarettes smoked during pregnancy at 1- and 6-months (plus a trend for this association at 1-week). The aforementioned variables were therefore controlled in further analyses.

Table 3.3

Spearman's Rho bivariate correlations (two-tailed) between breastfeeding duration and covariates at time point of the study.

Visit		Birth weight SDS	Household income	Maternal age	Maternal education	Cigarettes smoked during pregnancy	Age infant introduced to solids
1-week	<i>r</i>	-.02	.29**	.39***	.38***	-.21	
	<i>n</i>	81	81	81	81	81	
1-month	<i>r</i>	-.13	.33**	.41***	.43***	-.23*	
	<i>n</i>	77	77	77	77	77	
3-months	<i>r</i>	-.12	.15	.35**	.23*	-.15	
	<i>n</i>	74	74	74	74	74	
6-months	<i>r</i>	-.05	.27*	.43***	.38**	-.26*	.27*
	<i>n</i>	73	73	73	73	73	73
12-months	<i>r</i>	-.09	.14	.30*	.28*	-.12	.25*
	<i>n</i>	69	69	69	69	69	69

* $p < .05$ ** $p < .01$ *** $p < .001$

3.3.3 Breastfeeding and infant weight

One-tailed partial correlations were conducted to investigate the relationship between breastfeeding duration and weight SDS. Table 3.4 shows that (concurrent) breastfeeding duration is significantly negatively associated with infant weight SDS at 6- and 12-months and weight gain SDS from 1- to 6- and 1- to 12-months, but not with infant weight SDS at 1-week or 1-month. The effects seen here are substantial.

For example, a 6-month-old boy on the 50th centile weighs 8.05kg, a reduction of 1 SD at this time results in a weight of 7.74kg, representing a total reduction in weight of 0.32kg.

Table 3.4

Partial correlations (one-tailed) between concurrent breastfeeding duration and infant weight SDS controlling for maternal age, education, concurrent BMI, number of cigarettes smoked during pregnancy, household income, infant birth weight SDS and age introduced to solids.

	1-week SDS weight	1-month SDS weight	6-month SDS weight	12-month SDS weight	1- to 6- month SDS weight gain	1- to 12- month SDS weight gain
Breastfeeding Duration	-.02	-.16	-.33**	-.39**	-.31**	-.38**
<i>df</i>	64	64	64	60	63	59

* $p < .05$ ** $p < .01$

3.3.4 Breastfeeding and infant eating behaviours

One-tailed partial correlations were conducted to investigate the relationship between breastfeeding duration and infant eating behaviours at 12-months. Table 3.5 shows that after accounting for covariates, breastfeeding duration is significantly positively associated with slowness in eating, as measured by an age-appropriate version of the CEBQ.

Table 3.5

Partial correlations (one-tailed) between 12-month breastfeeding duration and infant eating behaviours (as reported by mothers using the CEBQ). Covariates include: maternal age, education, BMI, number of cigarettes smoked during pregnancy, household income, infant birth weight SDS and age introduced to solids.

	Satiety responsiveness	Enjoyment of food	Food responsiveness	Slowness in eating	Food fussiness	Desire to drink
Breastfeeding Duration	.12	-.08	-.11	.25*	.04	.01
<i>df</i>	60	60	60	60	60	60

* $p < .05$

3.3.5 Infant weight SDS and eating behaviours

One-tailed partial correlations were conducted to investigate the relationship between infant weight SDS and infant eating behaviours at 12-months. Table 3.6 shows that after accounting for covariates, infant weight SDS at 12-months is significantly negatively associated with satiety responsiveness and slowness in eating and significantly positively associated with enjoyment of food, as measured by an age-appropriate version of the CEBQ.

One-tailed partial correlations were then conducted between infant weight SDS and eating behaviours, which in addition to the above covariates, also controlled for breastfeeding duration. Table 3.7 shows that after accounting for breastfeeding duration in addition to the usual covariates, infant weight SDS at 12-months is significantly positively associated with enjoyment of food and significantly negatively associated with satiety responsiveness, but not slowness in eating, as measured by an age-appropriate version of the CEBQ.

Table 3.6

Partial correlations (one-tailed) between 12-month weight SDS and infant eating behaviours (as reported by mothers using the CEBQ). Covariates include: maternal age, education, BMI, number of cigarettes smoked during pregnancy, household income, infant birth weight SDS and age introduced to solids.

	Satiety responsiveness	Enjoyment of food	Food responsiveness	Slowness in eating	Food fussiness	Desire to drink
12-month weight SDS	-.30**	.28*	.21	-.26*	-.00	-.05
<i>df</i>	60	60	60	60	60	60

* $p < .05$ ** $p < .01$

Table 3.7

Partial correlations (one-tailed) between 12-month weight SDS and infant eating behaviours (as reported by mothers using the CEBQ). Covariates include: maternal age, education, BMI, number of cigarettes smoked during pregnancy, household income, infant birth weight SDS, age introduced to solids and breastfeeding duration.

	Satiety responsiveness	Enjoyment of food	Food responsiveness	Slowness in eating	Food fussiness	Desire to drink
12-month weight SDS	-.28*	.27*	.18	-.18	.01	-.05
<i>df</i>	59	59	59	59	59	59

* $p < .05$ ** $p < .01$

3.4 Discussion

This study aimed to investigate the relationships between breastfeeding duration and infant weight and eating behaviours during the first year of life. It was hypothesised that infants breastfed for longer durations would: (1) show slower weight gain between 1- and 6- and 1- and 12-months; (2) weigh less at 12-months than infants breastfed for shorter durations; and (3) demonstrate eating behaviours that are obesity-protective (as perceived and reported by their mothers) at 12-months. It was also hypothesised that infants who are heavier at 12-months will be reported as having more obesogenic eating behaviours by their mothers.

Results of the current study show that a longer duration of breastfeeding is associated with lower weight SDS at 6- and 12-months, but not at 1-week or 1-month, and slower weight gain from 1- to 6- and 1- to 12-months. Breastfeeding for longer durations is also associated with a slower rate of eating solid food, as perceived and reported by the mother. In addition, results also show that heavier infants are perceived by their mothers to: enjoy food more, be less satiety responsive and eat more quickly than leaner infants. Furthermore, results show that controlling for breastfeeding eradicates the association between infant weight and slowness in eating. One interpretation of this finding is that breastfeeding duration may be a mechanism by which lighter infants develop slower eating styles.

Although causality cannot be inferred due to the correlational nature of these analyses, Figure 3.1 illustrates these relationships in a hypothesised model. In the

model, it is hypothesised that breastfeeding encourages slower weight gain over the first postnatal year, which in turn leads to lower weight at 12-months. Breastfeeding may also encourage the infant to develop a slower rate of eating, which may then contribute to lower weight at 12-months. Breastfeeding may encourage a slower rate of eating due to breastfed infants having to work harder for their food than formula-fed infants (Cao et al., 2009). Breastfed infants may also better learn their internal cues of hunger and satiety (Birch & Fisher, 1998) than those fed formula milk.

However, it is also possible that infants who weigh less at 12-months may do so through having developed a slower rate of eating due to having a smaller appetite, rather than because of being breastfed. In addition, breastfeeding may not cause infants to eat more slowly. Instead, it may be the case that mothers of infants who are slow eaters may breastfeed for longer due to having a less demanding or hungry baby. Further research should clarify this in order to better understand breastfeeding's impact on eating behaviours and weight gain.

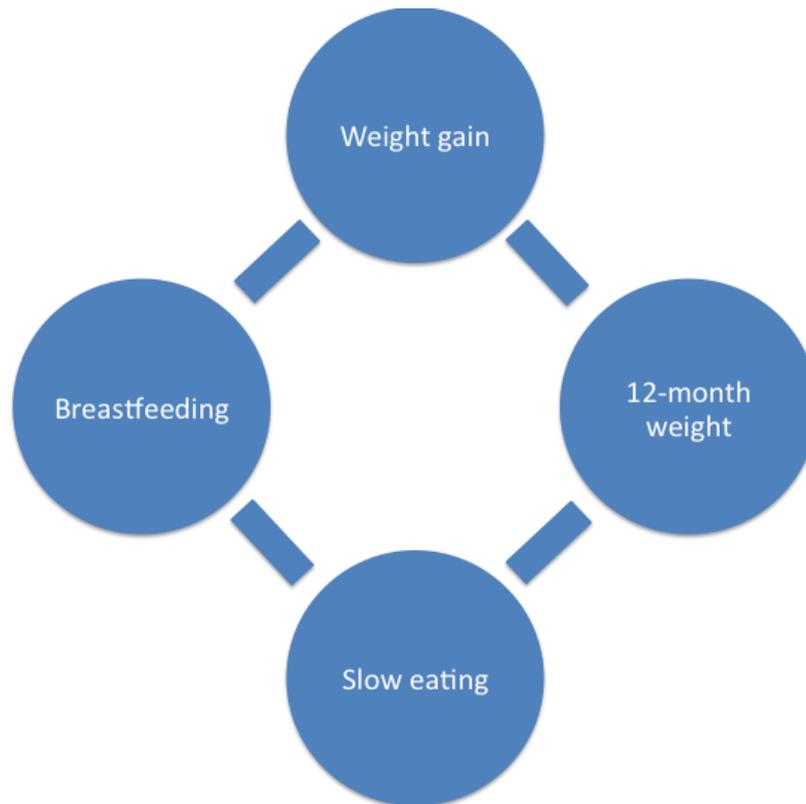


Figure 3.1

Model illustrating the significant relationships between breastfeeding duration, infant weight gain, weight and slow eating at 12-months.

Results of the current study show that breastfeeding duration is associated with lower infant weight at 6- and 12-months, but not at 1-week or 1-month after accounting for infant age and gender and other covariates. These results therefore suggest a dose-dependent effect of breastfeeding on infant weight and weight gain during the first year of life, which most clearly manifests in the latter half of the first year. These findings support those of Arenz et al., (2004), Harder et al., (2005), Hörnell et al., (2013), Kramer (1981), McCrory and Layte (2012) and Owen et al., (2005) who also found a dose-dependent effect of breastfeeding on later weight.

Results of the current study also found that breastfeeding is related to a slower rate of eating solid food, which indirectly supports Brown and Lee (2012). Although there was not a direct relationship found between breastfeeding duration and satiety responsiveness in infants, infants breastfed for shorter durations or not at all were reported to eat more quickly by their mothers and were heavier than those breastfed for longer. In-turn, heavier infants were reported as being less satiety responsive than lighter infants. Further research is required to establish whether breastfeeding helps infants develop obesity-protective eating behaviours. It is predicted that this may be the case due to the fact that breastfed infants have been found to adjust their intake according to the fat content of the milk (Tyson et al., 1992). It is also possible that introducing a bottle may speed-up feeding rate due to infants having to work less hard for their food.

Brown and Lee (2012) found that breastfeeding was related to satiety responsiveness when infants were 18- to 24-months-old. The lack of association between these variables in the current study could perhaps be due to the fact that infants were only 12-months-old. It would be of interest for future research to establish whether breastfeeding influences a slower rate of eating by 12-months, which in turn increases satiety responsiveness by 18- to 24-months. This is important, as previous research has found that greater satiety responsiveness is related to a lower risk of being overweight in childhood (Webber et al., 2009) and this is likely to be important in understanding breastfeeding's protective role against overweight and obesity in childhood.

One limitation of the current study is that a large number of questionnaires were distributed to participants at the 12-month home visit (see Chapter 2 for all measures given). This may have meant that participants became fatigued or bored and therefore could have completed the CEBQ less accurately. In addition, assessment of infant eating behaviours was based on maternal report. Mealtime observations of infants eating behaviour would more objectively assess eating behaviours that are deemed obesity-protective such as food and satiety responsiveness. Observations such as these would also allow investigation of maternal sensitivity and control during feeding, variables that have also been associated with infant weight gain and the development of eating behaviours (Farrow & Blissett, 2006b; Johnson & Birch, 1994).

The current study did not account for breastfeeding exclusivity, only duration. It is possible that if exclusivity was assessed in addition to duration, stronger relationships with eating behaviours may have been seen. In addition to this, although participants were from a variety of demographic, socioeconomic and cultural backgrounds, the educational level achieved by mothers in the current study was significantly higher than the national average (Office for National Statistics, 2011a). For example, from the 2011 census it can be seen that 27% of the population over 16-years-of-age had a Level 4 qualification or above (degree, higher degree or professional qualification), whereas 63% of the mothers in the current study had reached this level of education. Furthermore, although fewer women in the current study initiated breastfeeding compared to the national average (75% versus 81%), a higher proportion of women in the current study were breastfeeding at 6-months compared to the UK average (52% versus 34% [McAndrew et al., 2010a]). Although it is suggested that future

research should seek to recruit a sample more representative of the UK population, these factors were statistically controlled for in the current study's analyses, so their influence over the established relationships is not likely to be significant.

Another factor that requires more investigation is the bioactive factors present in breast milk. Leptin is one of these factors and research has found that it is implicated in weight gain and satiety responsiveness (Savino & Liguori, 2008). Cortisol is another hormone present in breast milk and it also has implications for weight gain, eating behaviours and maternal behaviour. A natural progression of this research will be to examine the interaction of breastfeeding, maternal sensitivity and control and biological predictors of adiposity, particularly those present in breast milk. This would allow a more thorough investigation of the predictors of weight gain and eating behaviours in infancy.

Despite inevitable limitations, the current study has several strengths. Firstly, the current study recruited a sample of women who had no complications, such as gestational diabetes, during pregnancy. Secondly, mother-infant dyads were visited at home several times over the first postnatal year; this promoted a good rapport between the researcher and participants and ensured accurate recording of breastfeeding duration and measurement of weight. Problems encountered by previous studies regarding retrospective self-report of information were therefore overcome. It is essential that any study intending to investigate breastfeeding's effect on infant weight gain and eating behaviours takes into account the aforementioned methodological shortcomings of previous research. The current study therefore

controlled for a range of covariates in analyses, including: maternal age, education, concurrent BMI, number of cigarettes smoked during pregnancy, household income, infant birth weight SDS and age introduced to solids. Furthermore, the sample was representative of the age of mothers giving birth in England and Wales. For example, in 2010, infants were most likely to be born to mothers between the ages of 25- and 34-years (Office for National Statistics, 2011b); the mean maternal age in the current study was 29.4 years.

The current study contributes to the published literature suggesting that breastfeeding is significantly associated with slower weight gain and lower weight at 6- and 12-months. This effect remains true even after controlling for covariates. Furthermore, breastfeeding is also likely to encourage the development of obesity-protective eating behaviours through learning to attend to their internal signals of hunger and satiety. Future research into breastfeeding and weight gain should move forward by incorporating investigation of maternal sensitivity and control during feeding alongside the bioactive factors in breast milk. Current breastfeeding rates are lower than that desired in the UK and so strategies to promote breastfeeding are important and could therefore result in significant benefits for health.

CHAPTER 4

CORTISOL METABOLISM AND WEIGHT ACROSS THE FIRST YEAR OF LIFE

Abstract

Background: Metabolism of cortisol is dysregulated in human adults with obesity. Furthermore, intrauterine exposure to glucocorticoids is associated with low birth weight and low birth weight is related to increased risk of abdominal adiposity later in life (Atkinson et al., 2005; Jaiswal et al., 2012). It is therefore essential to investigate relationships between cortisol metabolism and early infant weight gain so that early relationships between cortisol metabolism and infant adiposity can be better understood.

Method: Eighty-one mothers and infants were recruited from low-risk maternity units and 59 of these provided 24-hour nappy collections from infants and spot urine samples from themselves for the analysis of cortisol metabolism. Urine samples were collected at 1-week, 1-, 3-, 6- and 12-months postpartum. Participants were weighed and measured at home at 1-week, 1-, 6- and 12-months. Infant weight was converted to a standard deviation score (SDS), adjusted for age and gender.

Results: Partial correlations controlling for concurrent breastfeeding duration, infant weight SDS and maternal age and BMI revealed there was a significant positive association between infant and maternal ratios indicative of 5 α -reductase activity at 1-week, 1-, 6- and 12-months. Partial correlations controlling for maternal concurrent BMI, infant birth weight SDS, age introduced to solid food and concurrent

breastfeeding duration revealed that infant ratios indicative of less 11 β -HSD2 activity were significantly associated with slower weight gain from 1- to 12-months.

Discussion: The current study provides the first set of normative data on the cortisol metabolism of healthy human infants, showing that cortisol activation and clearance changes significantly over the first year of life, and by gender. Furthermore, the inverse relationship between 11 β -HSD2 activity and weight gain suggests that increased exposure to cortisol during the first year of life may limit growth.

4.1 Introduction

Cortisol is a glucocorticoid that maintains normal blood glucose levels and helps store excess glucose for future use. It is present in blood, urine and saliva (Cao et al., 2009) and has been related to obesogenic eating behaviours and overweight in human adults (Björntorp & Rosmond, 2000; Dallman et al., 2003; Marin et al., 1992; Pasquali et al., 1993; Tataranni et al., 1996 [see Chapter 1, Section 1.3.1 for a more detailed review of published literature]). Recent advances however, have led researchers to suggest that the overall level of serum cortisol may not be as important as the variations in cortisol metabolism in the investigation of weight and adiposity. For example, Vierhapper et al., (2004) found that although cortisol production rates were similar, metabolic clearance rates of cortisol were significantly higher in obese, than non-obese women. Furthermore, Rask et al., (2001) found that enhanced activation of cortisol in subcutaneous adipose tissue exacerbates obesity.

As described in Chapter 1, section 1.3.2, the enzymes responsible for the interconversion of cortisol and cortisone are 11 β HSD1, 11 β HSD2 and 5 α -reductase (see Figure 1.3 page 36 for a diagrammatic representation). 11 β HSD1 converts inactive cortisone into active cortisol; 11 β HSD2 inactivates cortisol into cortisone (Funder, 1985); and 5 α -reductase is an A-ring reductase, which enhances cortisol clearance by breaking it down into tetrahydro-metabolites (Raffaelli et al., 2006). 11 β HSD2 is located in mineralcorticoid tissues such as the kidney and the placenta; 11 β HSD1 is located in glucocorticoid tissues including liver, adipose and muscle (Tomlinson et al., 2007); and 5 α -reductase is expressed in skin, adipose tissue and liver (Russell & Wilson, 1994).

Research has demonstrated that the activity of 11 β HSD1 in human adults appears to be dysregulated in obesity (Rask et al., 2001; Stewart et al., 1999). Research has also found that 11 β -HSD2 activity is elevated in adiposity and is associated with insulin sensitivity in male and female human adults (Müssig et al., 2008). Furthermore, research has found that inactivation of cortisol by 5 α -reductase is increased and positively associated with insulin resistance in obese men and women (Andrew et al., 1998; Tomlinson et al., 2008) and BMI in obese and non-obese women (Vassiliadi et al., 2009). This increased inactivation of cortisol in human obesity occurs alongside a reduction in cortisol activation via 11 β HSD1 (Tomlinson et al., 2008). However, whilst it appears cortisol metabolism is associated with weight and adiposity in human adults, little is known about cortisol metabolism and infant weight and weight gain.

It is also important to investigate the possible relationships between infant weight gain and cortisol metabolism because whilst it is known that infants born of low birth weight are predisposed to metabolic disease and abdominal adiposity later in life (Atkinson et al., 2005; Jaiswal et al., 2012; Rinaudo & Wang, 2012), mechanisms underpinning this observation remain unspecified. However, it is known that being exposed to glucocorticoids in utero is associated with decreased birth weight of rat pups (Cleasby, Kelly, Walker & Seckl, 2003; Smith & Waddell, 2000), as well as lower birth weight, body length and head circumference (Braun et al., 2013) and hyperinsulinaemia in human neonates (Verhaeghe, van Bree, van Herck & Coopmans, 2005).

Research has also shown that 11 β -HSD2 has an important role in regulating foetal growth. Murphy et al., (2002) and Stewart et al., (1995) have found that lower activity of placental 11 β -HSD2 is associated with lower birth weight in human infants. In addition to this, McTernan et al., (2001) has shown that human neonates with intrauterine growth restriction have mothers who demonstrate lower placental 11 β -HSD2 expression. Reynolds (2010) supports aforementioned research suggesting that greater exposure to maternal cortisol in utero (through reduced clearance of it) limits intrauterine growth and changes gene expression and the structure and function of insulin target tissues. These changes programme adults for increased HPA axis activity, glucose intolerance, diabetes and obesity (Reynolds, 2010).

Research has also shown that deficits in 11 β -HSD2 can cause Apparent Mineralocorticoid Excess (AME) in humans, a syndrome that is associated with low

birth weight and presents with uncontrolled hypertension in childhood (Mune, Rogerson, Nikkilä, Agarwal & White, 1995). Hence, it is possible that individual variation in the development of early cortisol metabolism may be linked to variation in weight status in the first year of life. Previous research has emphasised the consequences of being exposed to excessive levels of glucocorticoids in utero in terms of later weight, adiposity and metabolic disease. These results reinforce the importance of further investigation of cortisol metabolism and early infant weight gain so that early relationships between cortisol metabolism and adiposity in infancy can be understood in more depth.

In fact, the developmental aspects of glucocorticoid metabolism in general require further examination (Dötsch et al., 2000). Currently, there is no published normative data on cortisol metabolism in human infants. Research has found significant associations between maternal and infant cortisol levels at 6- and 12-months postpartum (Stenius et al., 2008; Yehuda et al., 2005) but there is no published research investigating relationships between maternal and infant cortisol metabolism.

4.1.1 Aims and hypotheses

The current study sought to provide normative data on infant cortisol metabolism and infant weight gain. The aims of the study were to: i) explore the development of infant cortisol metabolism and examine whether a relationship exists between maternal and infant cortisol metabolism throughout the first postnatal year; and ii) investigate the

possible association between cortisol metabolism and bodyweight during the first year of life whilst controlling for covariates.

It was hypothesised that there would be a positive relationship between maternal and infant cortisol metabolism. It was also hypothesised that there would be an association between infant cortisol metabolism and bodyweight at 1-year-of-age. For example, it is possible that increased cortisol exposure through increased 11 β -HSD1, decreased 11 β -HSD2 or decreased 5 α -reductase activity, individually or in combination, might contribute to changes in weight gain across the first year of life.

4.2 Methods

4.2.1 Participants

Eighty-one full-term infants and their mothers were recruited from low-risk maternity units of the Birmingham Women's Hospital (see Chapter 2 for more information on recruitment and ethical approval). Of these 81 mother-infant dyads initially recruited, 59 mothers (mean age 29.47 years [SD 5.33]) and infants (36 males, 23 females; mean birth-weight 3.51 kg [SD 0.36]) provided urine samples for the analysis of cortisol metabolism.

Infants born prematurely (prior to 36-weeks gestation) or small for gestational age were not eligible for this study as these factors may be associated with weight gain during the first 12-months of life. Mothers who could not read or write English were not included.

4.2.2 Materials and procedure

Mothers were given information sheets on the maternity unit (Appendix 2 & 4) and consent was obtained for the researcher to telephone them to assess willingness to participate in the study (Appendix 3). Mothers happy to participate in the study were visited at home at approximately 1-week postpartum where consent forms were signed by the researcher and participant (Appendix 5).

Mothers and infants were visited at home when infants were aged approximately 1-week, 1-month, 6-months and 12-months. Mothers and infants were weighed and measured by the researcher at each visit. At 3-months, participants were contacted via telephone and urine samples were collected from those who had agreed to provide them. At each time point of the study mothers reported: feeding information (this included details regarding breast- and formula-feeding exclusivity and duration and when solids were first introduced); smoking and alcohol consumption; and any medications they were taking. Demographics were reported at the 1-week visit (see Table 2.1 in Chapter 2 for measures given at each home visit).

Demographic and Additional Information (Appendix 7)

Mothers completed a demographic and additional information sheet during the 1-week visit. Mothers reported age, pre-pregnancy weight, ethnic background, household income, educational level achieved and infant date of birth. Each mother also reported how she initially planned to feed her baby.

Additional Information (Appendix 8)

Mothers completed an additional information sheet, which requested information regarding medications currently being taken by the mother and present smoking and alcohol consumption.

Feeding Information (Appendix 10)

At each visit, mothers reported whether infants were being breast or formula-fed, and the duration and exclusivity of feeding method. At the later time points, questions were asked about introduction of solid foods and the types of foods infants were fed.

Anthropometric Measures

Infants were weighed naked with electronic scales by the researcher and weight was then converted to a standard deviation score (SDS), which adjusts measurements for age and gender (Child Growth Foundation, 1996). Mothers were weighed wearing light indoor clothing without shoes using electronic scales and maternal height was measured using a stadiometer.

Measurement and Analysis of Infant and Maternal Cortisol Metabolism

As explained in Chapter 2, mothers were asked to collect 24-hour samples of wet nappies at each time point during the study; full instructions were given to participating families (Appendix 4). Mothers were also requested to provide a single urine sample from themselves at the 1-week, 1-, 6- and 12-month home visits and if willing, a full 24-hour sample at the 3-month collection. The purpose of the 24-hour

collection was to attain a fuller picture of maternal cortisol metabolism for reference. Infant nappy and maternal urine samples were stored in freezers at the Institute of Biomedical Research at the University of Birmingham until they were ready for analysis.

Nappy collections were defrosted for 24-hours prior to the extraction process. The contents of the wet nappies were extracted in the lab using detailed instructions (Appendix 13) and records were kept throughout each extraction (Appendix 14). Once nappy extractions were completed, they were then analysed by a trained and reliable lab technician using GC/MS (see Appendix 15 for instructions). GC/MS is a method used for the analysis of metabolites of steroid hormones and a scanned run will detail every steroid excreted; it is superior to immunoassays regarding specificity, especially with low concentrations of the metabolites being assessed (Krone et al. 2010).

As described in Chapter 2, three ratios were calculated that reflected activity of 11 β -HSD1, 11 β -HSD2 and 5 α -reductase (see Figure 2.2, page 73):

- 1). The ratio of tetrahydro-metabolites of cortisol to those of cortisone (THF + 5 α THF/THE) provides a reflection of 11 β -HSD1 activity.
- 2.) The ratio of urinary cortisol to cortisone (F/E), reflects 11 β -HSD2 activity (Palermo, Shackleton, Mantero & Stewart, 1996).
- 3). The activities of 5 α -reductase were inferred from measuring the ratio of 5 α THF/THF.

4.2.3 Data analysis

Although steroid ratios were predominantly non-normally distributed (as indicated by Kolmogorov-Smirnov tests and histograms), some parametric tests were used where it was necessary to investigate the existence of possible relationships between variables whilst controlling for covariates because of a lack of a non-parametric equivalent test. Furthermore, Kolmogorov-Smirnov tests and histograms did indicate that infant weight and weight gain were normally distributed.

To test whether maternal steroid ratios change over time and whether infant ratios change over time and by gender, mixed effects models were used. Participants were measured for three different steroid ratios on five separate occasions. Therefore, the units of analysis (i.e., the values for each of the steroids) were hierarchically nested within time of measurement and then within participant. Such data sets often present with correlated errors that violate the inference assumptions of standard ANOVA and regression models. Therefore, these data required analysis using a mixed fixed and a random factor linear model, which use the random effects to model those sources of variation attributable to the design of the study. Statistical analysis was undertaken using the R software language (R Core Team, 2012), the mixed effects models were calculated using the LME4 package (Bates, Maechler & Bolker, 2012) and the Markov Chain Monte Carlo estimates of the random effects parameters and confidence intervals were calculated using the languageR package (Baayen, 2007).

Two-tailed non-parametric Spearman's rho correlations were used to assess whether demographic variables were associated with cortisol metabolism and weight. Household income, maternal education and quantity of cigarettes smoked during pregnancy were not related to infant or maternal weight or steroid ratios indicative of cortisol metabolism. These variables were therefore not controlled for in subsequent analyses. Maternal age was significantly associated with maternal steroid ratios indicative of 5α -reductase at 3-, 6- and 12-months and so was controlled for in analyses involving maternal ratios only ($p < .05$).

As cortisol is a hormone present in breast milk that is related to eating and weight (in human adults), concurrent breastfeeding duration was also controlled for in order to account for a possible additional influence of maternal cortisol metabolism on infant cortisol metabolism via breastfeeding. In addition to this, controlling for breastfeeding in these analyses also takes into account the effects breastfeeding has on growth, as discussed in Chapter 3, ensuring that our analysis of relationships between infant and maternal cortisol metabolism, and infant cortisol metabolism and growth, are not confounded by differences in breastfeeding patterns and durations.

One-tailed partial correlations (controlling for: concurrent breastfeeding duration, infant gender and weight SDS, maternal age and BMI) were used to investigate potential relationships between maternal and infant steroid ratios indicative of cortisol metabolism. To examine potential relationships between infant weight SDS and steroid ratios indicative of cortisol metabolism, two-tailed partial correlations

(controlling for: maternal concurrent BMI, concurrent breastfeeding duration, infant gender, birth weight SDS and age introduced to solid food) were performed.

4.3 Results

4.3.1 Descriptive statistics

Of the 81 mother-infant dyads initially recruited, 59 of them agreed to provide urine samples for the analysis of cortisol metabolism. Data from one infant was removed due to ratio levels being more than two standard deviations above the mean.

There were no differences in age between mothers who did ($M = 29.47$, $SE = 0.69$) and did not ($M = 29.27$, $SE = 1.55$) agree to provide urine samples $t(79) = 0.14$, $p = .91$. There were no differences in maternal education between those who did ($M = 6.02$, $SE = 0.32$) and did not ($M = 5.95$, $SE = 0.54$) provide urine samples $t(79) = 0.10$, $p = .92$. Furthermore, there were no differences in household income between families who did ($M = 4.86$, $SE = 0.23$) and did not ($M = 4.23$, $SE = 0.43$) agree to provide urine samples in this study $t(79) = 1.39$, $p = 0.17$.

There were no differences in the birth weight SDSs of infants whose mothers did ($M = 0.22$, $SE = 0.10$) and did not ($M = 0.35$, $SE = 0.19$) provide nappy samples, $t(79) = -0.64$, $p = .52$. There was also no difference in weight SDS at 12-months of infants whose mothers did ($M = -0.002$, $SE = 0.17$) and did not ($M = 0.18$, $SE = 0.25$) provide samples of nappies, $t(67) = -0.59$, $p = .56$. Finally, there was no difference in how long infants were breastfed between mothers who did ($M = 28.58$ weeks, $SE = 3.17$)

and did not ($M = 23.94$, $SE = 5.08$) provide urine and nappy samples, $t(67) = 0.78$, $p = .44$.

4.3.2 Maternal steroid ratios

Analysis involved using a mixed fixed and a random factor linear model, which used the random effects to model sources of variation attributable to the design of the study. In this model, time of measurement and type of steroid were treated as random factors within a random intercept model.

The interaction between time of measurement and type of steroid was evaluated against a null hypothesis model, which contained an intercept, main fixed effects for time of measurement and type of steroid and random effects for time of measurement and type of steroid. The null hypothesis model was significant ($\chi^2=16.373, df =6 p=0.01$) with an Akaike's Information Criterion of 565.21. The fixed effects of the null hypothesis model and Markov-chain Monte Carlo estimates of the random effects parameters and confidence intervals are shown in Table 4.1.

Table 4.1

Fixed effects of the null hypothesis model and Markov-chain Monte Carlo estimates of the random effects parameters and confidence intervals.

Fixed Effects					
	Wald Type II				
	Chi2	df	Pr(>Chi2)		
Time of Measurement	15.28	4	0.004		
Type of Steroid	10.73	2	0.005		
Random Effects					
	SD	MCMC median	MCMC mean	Lower 95% CI	Upper 95% CI
Time of Measurement	0.05	0.15	0.17	0	0.40
Type of Steroid	0.04	0.24	0.27	0	0.69
Residual	0.36	0.36	0.36	0.34	0.38

The fixed effect for the interaction between time of measurement and type of steroid was then added to the model and an ANOVA of the difference between the null hypothesis and the extended model was conducted. The fixed effects of the extended model and Markov-chain Monte Carlo estimates of the random effects parameters and confidence intervals are shown in Table 4.2.

Table 4.2

Fixed effects of the extended model and Markov-chain Monte Carlo estimates of the random effects parameters and confidence intervals.

Fixed Effects					
	Wald Type II Chi ²	<i>df</i>	Pr(>Chi ²)		
Time of Measurement	19.34	4	0.001		
Type of Steroid	13.59	2	0.001		
Time*Steroid	192.86	8	< 0.001		
Random Effects					
	SD	MCMC median	MCMC mean	Lower 95% CI	Upper 95% CI
Time of Measurement	0.04	0.12	0.14	0	0.35
Type of Steroid	0.03	0.15	0.19	0	0.51
Residual	0.32	0.32	0.32	0.30	0.33

The extended model produced a reduction in the Akaike's Information Criterion (AIC = 407.51) relative to the null hypothesis model. Therefore, the addition of the interaction between time of measurement and type of steroid significantly increased the explanatory value of the model ($X^2=173.69$, $df = 8$; $p < 0.001$). The time by steroid interaction was further explored by examining the effect of time of measurement on each of the types of steroid. The means, standard deviations and Wald Type II Chi-squared tests of deviation are reported in Table 4.3 and Figure 4.1 presents this data standardised as z-scores to aid comparison across the different types of steroid.

As can be observed from Table 4.3 and Figure 4.1, maternal steroid ratios F/E and THF+5 α THF/THE (indicative of 11BHS2 and 11BHS1 activity, respectively) do not appear to change significantly over the first postnatal year. However, the ratio 5 α THF/THF (indicative of 5 α -reductase activity) was very low in the immediate postpartum period; it increased throughout the first 3-months before slowing and then stabilising from 6- to 12-months. Ratios indicative of maternal 5 α -reductase activity therefore show significant changes over the course of the first postnatal year.

Table 4.3

Means, standard deviations and Wald Type II Chi-squared tests of deviation for the time by maternal steroid interaction.

Steroid	1-week		1-month		3-month		6-month		12-month		Chi		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Squared	df.	p
F/E	0.84	0.30	0.68	0.28	0.67	0.23	0.66	0.26	0.65	0.25	4.75	4	.31
THF+5 α THF/THE	0.88	0.22	0.81	0.21	0.88	0.24	1.01	0.27	0.82	0.21	5.84	4	.21
5 α THF/THF	0.20	0.11	0.47	0.34	0.86	0.44	1.04	0.60	0.99	0.46	37.76	4	<.001

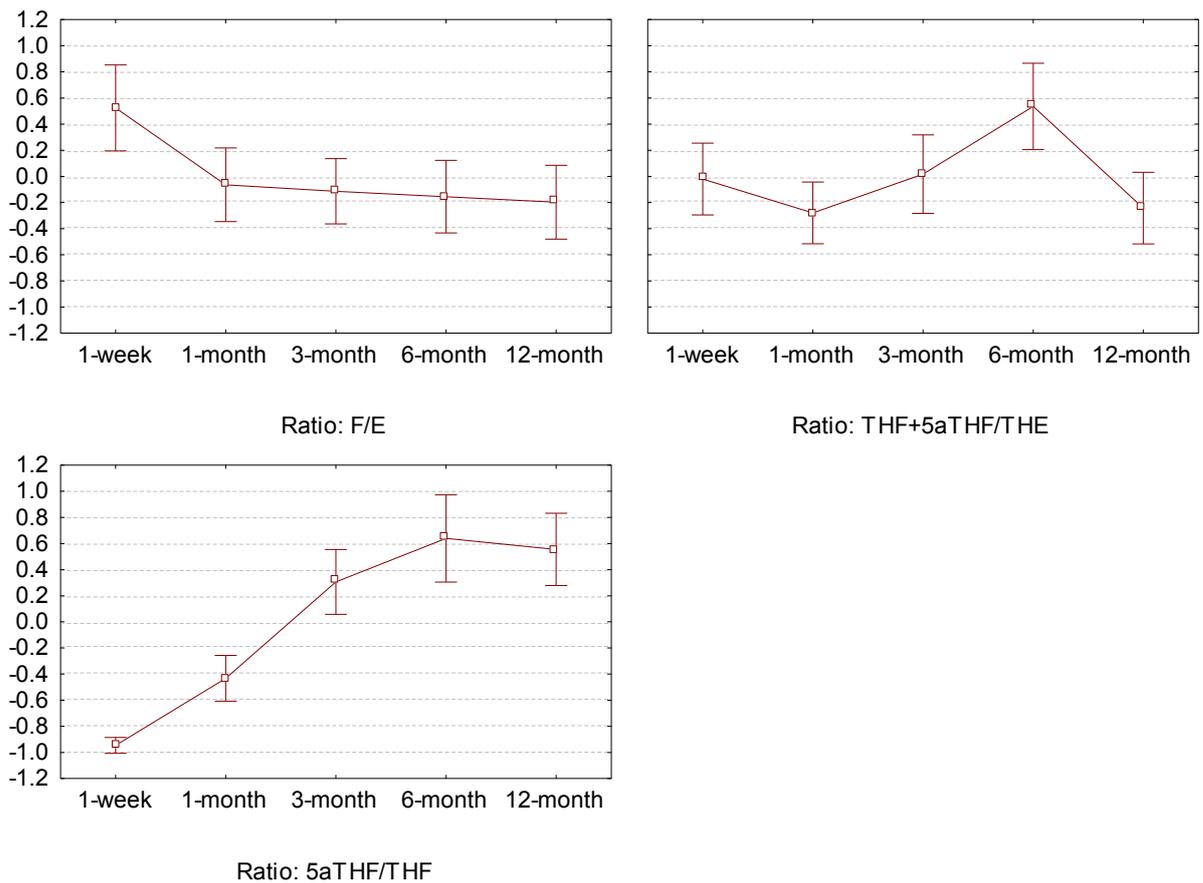


Figure 4.1

Mean Z-Scores and 95% confidence intervals for the time by maternal steroid interaction.

4.3.3 Infant steroid ratios

As with the aforementioned maternal data, infants were measured for three different steroid ratios on five separate occasions. Data was modelled using a mixed fixed and a random factor linear effects model, which used random effects to model those sources of variation attributable to the design of the study. In this model time of measurement and type of steroid were treated as random factors within a random intercept model.

The interaction between time of measurement, type of steroid and gender was evaluated against a null hypothesis model, which contained an intercept, main fixed effects for time of measurement, type of steroid and gender and random effects for time of measurement and type of steroid. The null hypothesis model was significant ($\text{Chi}^2 = 36.59$, $df = 7$, $p < 0.001$) with an Akaike's Information Criterion of 2897.0. The fixed effects of the null hypothesis model and Markov-chain Monte Carlo estimates of the random effects parameters and confidence intervals are shown in Table 4.4.

Table 4.4

Fixed effects of the null hypothesis model and Markov-chain Monte Carlo estimates of the random effects parameters and confidence intervals.

Fixed Effects					
	Wald Type II Chi ²	df	Pr(>Chi ²)		
Time of Measurement	49.00	4	P<0.001		
Type of Steroid	239.19	2	P<0.001		
Random Effects					
	SD	MCMC median	MCMC mean	Lower 95% CI	Upper 95% CI
Time of Measurement	0.20	0.48	0.59	0	1.58
Type of Steroid	0.16	1.02	1.29	0	3.52
Residual	1.54	1.54	1.54	1.46	1.62

The fixed effect for the two-way and three-way interactions between time of measurement, type of steroid and gender was then added to the model and an ANOVA of the difference between the null hypothesis and the extended model was conducted. The fixed effects of the extended model and Markov-chain Monte Carlo

estimates of the random effects parameters and confidence intervals are shown in Table 4.5.

Table 4.5

Fixed effects of the extended model and Markov-chain Monte Carlo estimates of the random effects parameters and confidence intervals.

Fixed Effects					
	Wald Type II Chi ²	df	Pr(>Chi ²)		
Time of Measurement	87.45	4	< 0.001		
Type of Steroid	426.88	2	< 0.001		
Gender	12.07	1	< 0.001		
Time*Steroid	561.37	8	< 0.001		
Time*Gender	20.07	4	< 0.001		
Steroid*Gender	16.18	2	< 0.001		
Time*Steroid* Gender	32.2	8	< 0.001		
Random Effects					
	SD	MCMC median	MCMC mean	Lower 95% CI	Upper 95% CI
Time of Measurement	0.20	0.45	0.59	0	1.79
Type of Steroid	0.16	1.11	1.38	0	3.84
Residual	1.54	1.54	1.54	1.46	1.61

The extended model produced a reduction in the Akaike's Information Criterion (AIC = 2449.3) relative to the null hypothesis model. Therefore, the addition of the interactions between time of measurement type of steroid and gender significantly increased the explanatory value of the model ($X^2=469.16$, $df = 16$; $p < 0.001$).

The time by steroid interaction was further explored by examining the effect of time of measurement on each of the types of steroid. The means, standard deviations and Wald Type II Chi-squared tests of deviation are reported in Table 4.6 and this data standardised as z-scores to aid comparison across the different steroids is presented in Figure 4.2.

As can be observed from Table 4.6 and Figure 4.2, there was a significant difference in infant steroid ratios between boys and girls across the first year of life. 11β -HSD1 activity, reflected by the THF+ 5α THF/THE ratio, was almost undetectable until 3-months-of-age and then increased dramatically between 3- and 6-months with stable values at 12-months ($p < .001$). Boys had significantly higher ratios indicative of 11β -HSD1 activity than girls at 3-months, but not at any other time point during the study.

Paralleling these observations, infant 5α -reductase activity, as measured by the ratio 5α THF/THF, was also low at birth, rose significantly to a peak at 3-months of age and then decreased at 6-months with a further decrease at 12-months of age ($p < .001$). Boys had significantly higher ratios indicative of 5α -reductase activity at 3-months than girls, but not at any other time point during the study.

The F/E ratio also increased with time, which indicates a 11β -HSD2 activity decreased over the first year of life. Although this pattern was less dramatic than that observed with 11β -HSD1 and 5α -reductase activity (Figure 4.2), overall the ratio increased significantly between 6- and 12-months. There were no significant

differences between male and female infants in this ratio indicative of 11β -HSD2 activity at any time point during the first year of life.

Table 4.6

Means, standard deviations and Wald Type II Chi-squared tests of deviation for the time by infant steroid interaction.

Steroid	1-week		1-month		3-month		6-month		12-month		Chi		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Squared	df	p
F/E	0.18	0.99	-0.52	0.71	-0.30	0.43	-0.13	0.65	0.93	1.37	23.20	4	<0.001
THF+5 α THF/THE	-0.85	0.01	-0.80	0.05	-0.08	0.70	0.96	1.07	0.96	0.55	117.29	4	<0.001
5 α THF/THF	-1.07	0.19	-0.12	0.74	1.05	1.07	0.38	0.72	-0.35	0.37	71.31	4	<0.001

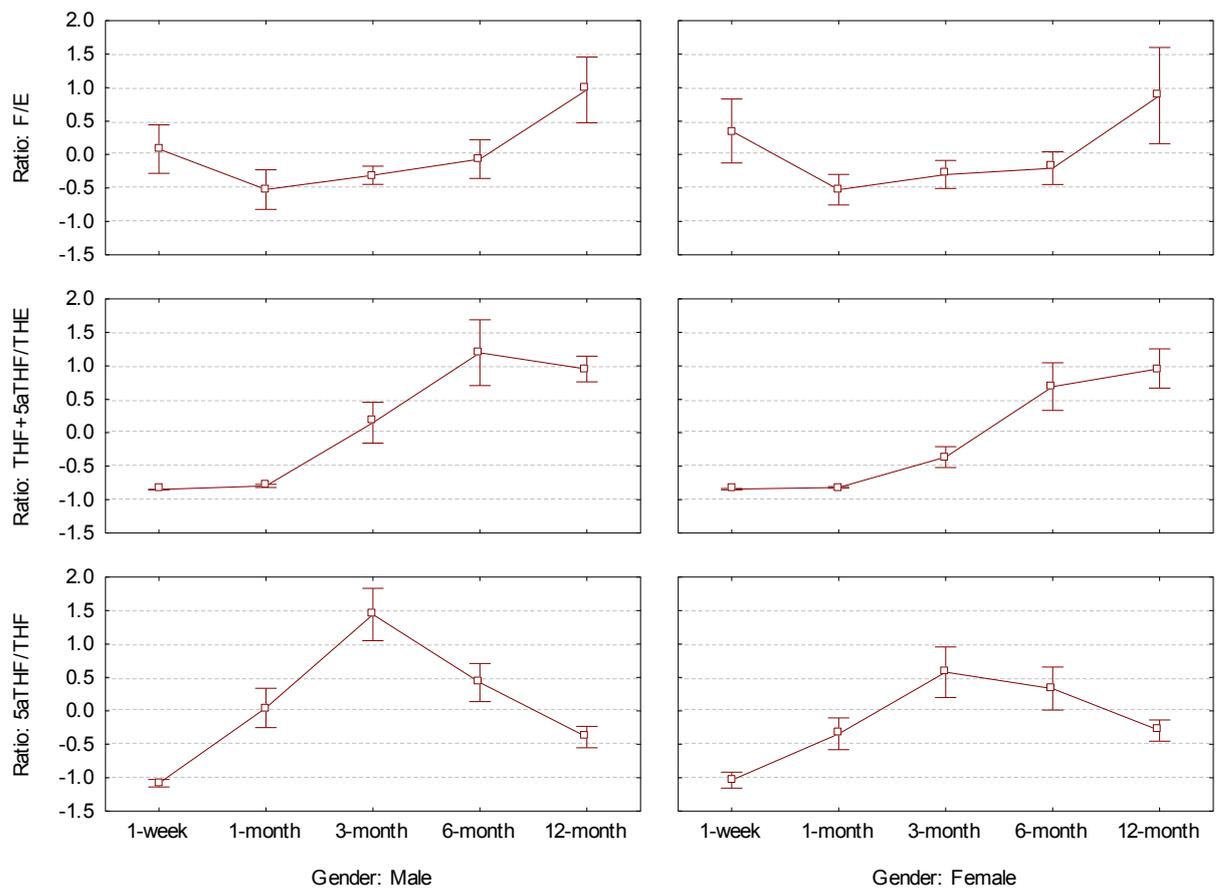


Figure 4.2

The mean Z-Scores and 95% confidence intervals for the time by infant steroid interaction.

4.3.4 Infant and maternal cortisol metabolism

One-tailed partial correlations were performed adjusting for confounding variables (including infant gender and weight SDS and maternal age, BMI and concurrent breastfeeding duration) to examine any potential relationships between the maternal and infant steroid ratios indicative of cortisol metabolism. As can be seen in Table 4.7, there were significant positive correlations showing a medium effect between infant and maternal 5αTHF/THF (indicative of 5α-reductase activity) at every time point of the study.

There was also a significant negative correlation showing a medium effect between infant F/E (indicative of 11BHSD2 activity) and maternal 5α THF/THF (indicative of 5α -reductase activity) at 1-week, but not at 1-, 3-, 6- or 12-months. There was a significant positive correlation showing a small effect between infant THF+ 5α THF/THF (indicative of 11BHSD1 activity) and maternal 5α THF/THF (indicative of 5α -reductase activity) at 1-month, but not at 1-week, 3-, 6- or 12-months. Furthermore, there was also a significant positive correlation showing a small effect between infant and maternal F/E (indicative of 11BHSD2 activity) at 6-months, but not at 1-week, 1-, 3- or 12-months.

Table 4.7

Partial correlations (one-tailed) between infant and maternal steroid ratios F/E, THF+5 α THF/THE and 5 α THF/THF (indicative of 11BHS2, 11BHS1 and 5 α -reductase), controlling for: concurrent breastfeeding duration, infant gender and weight SDS and maternal age and BMI.

		Maternal														
		1-week df = 38			1-month df = 46			3-months df = 39			6-months df = 41			12-months df = 34		
		F/E	THF+5 α THF/THE	5 α THF/THF	F/E	THF+5 α THF/THE	5 α THF/THF	F/E	THF+5 α THF/THE	5 α THF/THF	F/E	THF+5 α THF/THE	5 α THF/THF	F/E	THF+5 α THF/THE	5 α THF/THF
Infant	Concurrent F/E	-.18	-.14	-.36*	-.04	-.05	-.03	.05	-.13	-.07	.26*	-.07	-.08	.14	.00	-.21
	Concurrent THF+5 α THF/THE	-.15	.02	-.07	-.09	-.07	.26*	.01	.12	.21	.05	-.08	-.02	.03	.13	-.05
	Concurrent 5 α THF/THF	-.24	-.02	.34*	-.11	-.11	.31*	-.08	.18	.32*	-.07	-.10	.28*	-.02	.20	.33*

* $p < .05$ ** $p < .01$

The above partial correlations were run again, to investigate relationships between maternal and infant steroid ratios, by feeding method. However, as only two participants were formula-fed from birth and only two participants were breast- and formula-fed from birth, only analyses with infants exclusively breastfed from birth are presented. Furthermore, partial correlations only used 1-week, 1-month and 3-month data, as infants would have been introduced to solid food by 6-months, thus rendering the question as no longer valid. Infants who had already been introduced to solid food by 3-months were excluded from this analysis.

As can be seen from Table 4.8, there was a significant negative correlation showing a medium effect between infant and maternal F/E (indicative of 11BHS2 activity) at 1-week, but not at 1-, or 3-months. At 1-month, there was a significant negative correlation showing a moderate effect between infant and maternal THF+5 α THF/THF (indicative of 11BHS1 activity), but not at 1-week or 3-months. There was also a significant negative correlation showing a moderate effect between infant 5 α THF/THF (indicative of 5 α -reductase activity) and maternal THF+5 α THF/THF (indicative of 11BHS1 activity) at 1-month, but not at 1-week or 3-months. Interestingly, the correlations (seen in Table 4.7) between infant and maternal 5 α THF/THF were no longer present. This suggests that the relationship between infant and maternal 5 α -reductase activity may be more influenced by external factors, than by the contents of breast milk.

Table 4.8

Partial correlations (one-tailed) between infant (exclusively breastfed) and maternal steroid ratios F/E, THF+5 α THF/THE and 5 α THF/THF (indicative of 11BHS2, 11BHS1 and 5 α -reductase), controlling for: infant gender and weight SDS and maternal age and BMI.

		Maternal								
		1-week			1-month			3-months		
		F/E	THF+5 α THF/THE	5 α THF/THF	F/E	THF+5 α THF/THE	5 α THF/THF	F/E	THF+5 α THF/THE	5 α THF/THF
	df	19	19	19	19	19	19	17	17	17
	Concurrent F/E	-.40*	.05	-.13	-.07	-.02	.15	.11	-.15	-.21
Infant	Concurrent THF+5 α THF/THE	.03	.11	-.13	.01	-.39*	.25	.18	-.21	-.13
	Concurrent 5 α THF/THF	.10	-.16	.16	-.12	-.50*	.30	-.06	-.27	-.10
		* $p < .05$			** $p < .01$					

4.3.5 Infant weight and cortisol metabolism

Two-tailed partial correlations were performed adjusting for confounding variables (maternal concurrent BMI, infant birth weight SDS, age introduced to solid food and concurrent breastfeeding duration) to examine any potential relationships between infant weight SDS and steroid ratios indicative of cortisol metabolism. To reduce the number of analyses, and therefore the risk of finding significant results due to multiple testing, only concurrent ratios were considered.

As can be seen in Table 4.9, there was a trend for a negative relationship between infant weight SDS at 12-months and concurrent ratios indicative of 11 β -HSD2 activity ($p = .07$). In addition to this, there was a significant negative correlation showing a medium effect between infant weight gain SDS from 1- to 12-months and steroid ratios indicative of 11 β -HSD2 activity. For every increase in 1 SD (0.34) of F/E at 12-months (indicative of reduced 11 β -HSD2 activity), 1- to 12-month weight gain SDS slows down by 0.37. Therefore, for every 0.34 increase in F/E, weight gain slows down by more than a third.

Table 4.9

Partial correlations (two-tailed) between infant weight SDS and concurrent steroid ratios indicative of 11BHSD2, 11BHSD1 and 5 α -reductase, controlling for: maternal concurrent BMI, infant gender, birth weight SDS and age introduced to solid food and concurrent breastfeeding duration.

	<i>df</i>	Concurrent 11BHSD2	Concurrent 11BHSD1	Concurrent 5 α -reductase
1-week weight SDS	39	-.14	.22	-.08
1-month weight SDS	46	-.08	.10	.02
6-month weight SDS	43	.09	.00	.04
12-month weight SDS	41	-.28	.15	.07
1- to 6-month weight gain SDS	43	.00	-.05	.08
1- to 12-month weight gain SDS	41	-.31*	.15	.07
6- to 12-month weight gain SDS	41	-.24	.13	.17

* $p < .05$ ** $p < .01$

4.4 Discussion

This study aimed to explore the development of infant cortisol metabolism and whether a relationship exists between maternal and infant cortisol metabolism throughout the first postnatal year. This study also aimed to investigate the possible association between cortisol metabolism and bodyweight during the first year of life. It was hypothesised that there would be a positive relationship between maternal and infant cortisol metabolism. It was also hypothesised that that there would be an association between infant cortisol metabolism and bodyweight at 1-year-of-age.

Results of the current study show that maternal steroid ratios indicative of 5α -reductase activity show significant changes over the course of the first postnatal year; 5α -reductase activity appeared very low at 1-week postpartum and then increased throughout the first 3-months before slowing and then stabilising from 6- to 12-months. Maternal steroid ratios indicative of 11β HSD2 and 11β HSD1 activity did not appear to change over the first postnatal year. Research has not yet shown how changes in 5α -reductase occur, or what these changes may mean. However, 5α -reductase activity is positively associated with weight change and insulin resistance and it affects cortisol availability (Andrew et al., 1998; Nelson, Legro, Strauss, McAllister, 1999; Schmidt et al., 2006; Tomlinson et al., 2008; Vassiliadi et al., 2009). Although causality has not been inferred among these associations, it is possible that the changes in maternal 5α -reductase activity in the current study may be associated with the changes in weight that occur postnatally. This thesis has provided the first normative set of data describing maternal cortisol metabolism, in a non-clinical sample, throughout the immediate postnatal period and first postpartum year.

When analysing infant steroid ratios, results show that there are significant differences over time and between male and female infants across the first year of life. Ratios indicative of cortisol activation (via 11β -HSD1) increased dramatically between 3- and 6-months and stabilised around 12-months. Ratios indicative of cortisol clearance (via 5α -reductase) peaked at 3-months before decreasing at 6- and 12-months. In addition, boys appeared to be both activating and clearing more cortisol than girls at 3-months, but not at any other time point. Conversely, infant steroid ratios indicated that cortisol clearance via 11β -HSD2 decreased over the first

year of life and most significantly between 6- and 12-months. There were no significant gender differences in 11 β -HSD2 activity at any time point during the first year of life.

Previously it has been stated that developmental aspects of glucocorticoid metabolism require more investigation (Dötsch et al., 2000). Changes in steroid ratios result in changes in metabolic phenotype and alterations to weight and insulin sensitivity in human adults (Tomlinson et al., 2008). It cannot be concluded from this study whether this is also the case during infancy, although it is possible. Full examination of metabolic phenotype during infancy, would have required blood samples to measure insulin and glucose. Despite this however, this study is the first to have provided normative data on cortisol metabolism in human infants. Furthermore, it is the first data set to show gender differences in ratios indicative of cortisol activation (via 11 β -HSD1) and clearance (via 5 α -reductase, [but not 11 β -HSD2]) in human infants during the first year of life.

Results of the current study also show that after controlling for effects of infant gender and weight SDS, maternal age and BMI and concurrent breastfeeding duration, infant ratios indicative of more 5 α -reductase activity were associated with maternal ratios also indicative of more 5 α -reductase activity throughout the first postnatal year. Therefore, mothers who clear more cortisol have infants who clear more cortisol too. Results also indicate that mothers who clear more cortisol via 5 α -reductase have infants who clear more cortisol via 11 β HSD2 at 1-week, but not at any other time point. In addition, mothers who clear more cortisol via 5 α -reductase

have infants who activate more cortisol at 1-month, but not any other time point. Furthermore, mothers who clear more cortisol via 11BHS2 have infants who also clear more cortisol via 11BHS2 at 6-months.

Earlier research has found relationships between infant and maternal cortisol levels at 6- and 12-months postpartum (Stenius et al., 2008; Yehuda et al., 2005). However this study is the first to have looked at relationships between infant and maternal cortisol metabolism in a sample of healthy mothers and their healthy infants from the neonatal period to one year of age. Interestingly, these relationships remain true even when breastfeeding is controlled for – a feeding method likely to expose the infant to maternal levels of cortisol. Associations between infant and maternal cortisol metabolism could therefore be due to genetic or environmental factors, as they do not appear to be due to the exposure of maternal cortisol through breast milk.

Findings from the current study also indicate that after controlling for effects of concurrent breastfeeding duration, maternal concurrent BMI, infant gender, birth weight SDS and age introduced to solid food, steroid ratios indicative of less 11 β -HSD2 activity are significantly associated with slower weight gain from 1- to 12-months. There was also a trend indicating a relationship between infant 11 β -HSD2 activity and weight at 12-months.

Published research relating 11 β -HSD2 to growth and weight has found that 11 β -HSD2 is essential in regulating foetal growth. Being exposed to greater levels of cortisol in utero, through reduced clearance rates of it via 11 β -HSD2 in the placenta,

is associated with lower birth weight in human infants (Murphy et al., 2002; Stewart et al., 1995). Furthermore, the condition AME is associated with low birth weight and is caused by deficits in 11 β -HSD2. Results of the current study are the first to demonstrate that lower 11 β -HSD2 activity is associated with slower postnatal weight gain from 1- to 12-months. The current research therefore suggests that greater exposure to cortisol in the postnatal period (through reduced clearance of it) is associated with slower weight gain. Future research should establish if and what the longer-term effects of this association might be.

Interestingly, the association between lower 11 β -HSD2 activity and slow weight gain from 1- to 12-months remains true even after controlling for breastfeeding duration. Given that Chapter 3 found breastfeeding duration is associated with slower weight gain during the first year of life, the results of the current chapter suggest that breastfeeding's effects on weight do not appear to be strongly mediated by cortisol. This will be investigated further in Chapter 6.

One limitation of the current study is that it cannot be guaranteed that all nappy samples were 24-hour collections. Firstly, only wet nappies were suitable for analysis, mothers were requested to dispose of soiled nappies. Secondly, mothers may have forgotten to start collecting nappies and so some may have been 'missed'. However, in the attempt to prevent this being an issue, participants were reminded to start collecting nappies the day before the visit took place. Thirdly, mothers may have supplied nappies, unknowingly, that were unusable. Nappies were generally found

unusable when they were slightly soiled or when they did not contain enough urine to be extracted.

Another limitation is that not all participants provided urine samples at each visit (see Appendix 18). This may have been either because their infant was unwell or because their infant had a nappy rash and so needed to use a cream to treat this. Creams required to treat nappy rash interfere with steroid metabolism analysis and so participants needing to use such creams could not provide nappy samples. In order to prevent these issues occurring, the mothers were offered an alternative collection day.

Despite these limitations, it is important to recognise the strengths of the current study. Firstly, the design of the study meant that because mothers were approached on maternity units and subsequently visited at home, a good rapport between the researcher and participants was established quickly and this helped ensure the accurate collection of samples and other data. Secondly, the procedure of collecting samples for the analysis of cortisol metabolism was not an invasive one. It is likely that obtaining maternal consent for collecting blood samples would have been considerably more difficult. Finally, the current study recruited a sample of women who had no complications, such as gestational diabetes, during pregnancy and infants were born full-term of a healthy weight. No medical conditions were reported for mother or infant and it can therefore be said with confidence that the current study has achieved its aim in providing the first set of normative data on the cortisol metabolism of healthy human infants.

Future research should seek to investigate cortisol metabolism and weight gain in premature and low birth weight infants. Such infants can show rapid catch-up growth in early infancy and rapid weight gain during this time has been found to predict obesity later in life (Cole, 2007; Lanigan & Singhal, 2009; Taveras et al., 2009). Investigating cortisol metabolism and weight gain in premature and low birth weight infants is therefore likely to enhance understanding of the contributors to excessive weight in infancy. It may also help increase the knowledge base required to develop effective preventions and interventions of obesity.

Mothers have been described as being organisers of their infants' psychobiological function in the first year of life (Spangler et al., 1994). Furthermore, it has been found that mothers who have anxiety and depression have infants who produce more cortisol, have higher cortisol reactivity and less mature regulatory behaviours (Azak et al., 2013; Feldman et al., 2009; Grant et al., 2009) than the infants of non-depressed mothers. Given published findings on cortisol metabolism and weight in human adults, interactions between maternal behaviour, infant cortisol metabolism, infant eating behaviours and growth warrant more investigation. The next stage of this research is to incorporate investigation of relationships between maternal and infant behaviours.

In conclusion, the current study has demonstrated that cortisol activation and clearance changes significantly over the first year of life, and by gender. Furthermore, there is an inverse relationship between 11β -HSD2 activity and weight gain across the first year of life, suggesting that increased exposure to cortisol may

limit growth. Further studies are essential to establish whether this relationship is maintained later in childhood, and to understand its relevance in children that were born premature or small for gestational age.

CHAPTER 5

BREASTFEEDING DURATION, POSITIVE MATERNAL INTERACTIONS AND CORTISOL METABOLISM

Abstract

Background: Previous research has found that longer durations of breastfeeding are associated with increased maternal sensitivity and faster cortisol recovery in infants. The current study aimed to investigate potential associations between breastfeeding duration, positive maternal interactions during feeding and infant cortisol metabolism at 6- and 12-months-of-age, whilst accounting for key confounding variables and utilising observational methods of maternal behaviour.

Method: Eighty-one mothers and their infants were recruited from low-risk maternity units; 59 of these provided 24-hour nappy collections from infants for the analysis of cortisol metabolism at 1-week, 1-, 3-, 6- and 12-months. 58 mother-infant dyads were observed feeding their infant at home at 6-months and 55 at 12-months. Observations were coded for maternal vocalisations and appropriateness and sensitivity of behaviour during the mealtime.

Results: Partial correlations (controlling for: maternal age, education, number of cigarettes smoked during pregnancy, household income and infant age introduced to solids) revealed positive associations between breastfeeding duration and observed maternal vocalisations, appropriateness and sensitivity during a mealtime at 12-months postpartum. Partial correlations (controlling for: maternal age, education, number of cigarettes smoked during pregnancy, household income and infant weight

SDS and age introduced to solids) revealed positive associations between: (1) Infant 11 β -HSD1 activity and maternal appropriateness at 12-months; (2) Infant 11 β -HSD1 activity and breastfeeding duration at 12-months; (3) Infant 5 α -reductase activity and maternal vocalisations at 12-months; and (4) Infant 5 α -reductase activity and breastfeeding duration at 6- and 12-months. Controlling for breastfeeding removed the association between maternal interactions and infant cortisol activation, but not clearance.

Discussion: The current study is the first to investigate relationships between breastfeeding duration, positive maternal mealtime interactions and infant cortisol metabolism throughout the first year of life. Results indicate that infants who are breastfed for longer durations activate and clear more cortisol at 12-months. It is also suggested that whilst breastfeeding duration appears to explain some of the relationship between infant 11 β -HSD1 activity and positive maternal interactions, there are significant aspects of maternal behaviour, other than breastfeeding duration, that are involved in cortisol clearance in infants.

5.1 Introduction

As discussed in Chapter 1, breastfeeding has a protective dose-dependent effect on overweight and obesity in childhood and adolescence (Arenz et al., 2004; Hörnell et al., 2013; Kramer, 1981; McCrory & Layte, 2012; Owen et al., 2005). Results of Chapter 3 suggest that a longer duration of breastfeeding is associated with slower weight gain throughout the first postnatal year and lower weight at 6- and 12-months. However, the mechanisms involved in this relationship require further research.

There is a variety of potential mechanisms to consider, which include: the biologically active ingredients within breast milk (Miralles et al., 2006); facilitation of maternal sensitivity (Brown & Lee, 2013; DiSantis et al., 2013); maternal experience of allowing the infant to control its own intake (Birch & Fisher, 1998); the lower caloric and protein content of breast milk versus formula milk (Alexy et al., 1999); and the fact that breastfed infants may be able to better attend to internal signals of hunger and satiety (Birch & Fisher, 1998).

Maternal sensitivity is one of the aforementioned potential mechanisms and research has found it to be positively associated with breastfeeding duration (Tharner et al., 2012). Maternal sensitivity refers to how accurately mothers perceive and interpret their infant's signals and how prompt and appropriate their responses are to these communications (Ainsworth et al., 1979). Britton, Britton and Gronwaldt (2006) found that mothers who breastfeed exhibit greater sensitivity towards their infants at 3-months postpartum than those who do not. Additionally, a longer duration of breastfeeding is associated with higher sensitivity scores during the first year of life (Britton et al., 2006). Breastfeeding mothers have also been found to demonstrate higher quality interactions with their infants at 12-months, regardless of their level of household income (Gutman, Brown & Akerman, 2009). However, it is not really known whether breastfeeding facilitates maternal sensitivity or whether more sensitive mothers choose to breastfeed and breastfeed for longer.

Sensitivity during feeding is essential because insensitive mothers may not register, or may even ignore, infant signals, which could then override the infant's internal

hunger and satiety cues. Maternal sensitivity, particularly during feeding, may therefore be one of the mechanisms by which breastfeeding protects against obesity later in life. To further develop this area of research, it would be of particular interest to look at the interaction of breastfeeding and maternal sensitivity along with biological predictors of adiposity, particularly those present in breast milk. A study of this nature will allow more detailed exploration of the relationships between infant feeding, maternal behaviours and infant physiology.

Maternal behaviour coordinates infant psychobiological function during the first year of life (Spangler et al., 1994). Animal models have shown that early maternal care has a functional effect on the HPA axis in infants, suggesting that maternal behaviour may have an organisational effect on infant stress regulation responses and glucose regulation (Murray et al., 2010). Further to this, Hofer (1994) suggested that within parent-child interactions are sensorimotor, thermal and nutrient-based events and these events have extensive effects on the physical and behavioural development of the infant. Hofer explains that being deprived of these regulators causes dysregulation of the infant's stress response. Examining breastfeeding in this context therefore assumes that the parent-child interaction during breastfeeding aids infant physiological and behavioural development and regulation of his or her stress response.

One biologically active ingredient of breast-milk that has previously been associated with infant and maternal behaviour is cortisol. Beijers et al., (2013) found that a longer duration of breastfeeding predicted faster cortisol recovery in infants after

being exposed to a psychological stressor; it was concluded that breastfeeding positively contributes to infant cortisol regulation. Results imply that breastfeeding for longer is associated with a faster recovery from stress. It was suggested that as persistently elevated levels of cortisol can have negative effects on HPA-axis functioning, faster cortisol recovery may be interpreted as an adaptive response. Further to this, Grant et al., (2009) found that maternal insensitivity is an additive and independent predictor of 7-month-old infants' cortisol reactivity in response to a stressful situation.

In addition to infant cortisol regulation, research has also investigated relationships between breastfeeding and maternal cortisol levels. Patacchioli et al., (1992) found that in the immediate postnatal period, breastfeeding does not appear to affect maternal cortisol levels. Conversely, when investigating the more long-term effects, Lankarani-Fard, et al., (2001) found that mothers who breastfed for longer than 12-months had significantly higher cortisol levels between the ages of 50- and 89-years. Given that breastfeeding may influence maternal cortisol levels and that cortisol levels appear to be involved in maternal sensitivity to infant cues (Fleming et al., 1987 & 1997), it is of interest to explore potential relationships between breastfeeding duration and maternal metabolism of cortisol.

As highlighted in Chapters 1 and 4, cortisol is involved in the maintenance of blood glucose levels and the storage of glucose for future use; it also has implications for weight gain. Advances in this area of research have led to the suggestion that investigating the metabolism of cortisol, rather than the level of cortisol, may be more

important in the examination of predictors of weight gain. For example, rates of cortisol clearance and production have been found to be higher in obese individuals whilst circulating levels often appear normal (Andrew et al., 1988; Björntorp & Rosmond, 2000; Vierhapper et al., 2004). It is important to note here that no study has yet investigated cortisol metabolism with regard to breastfeeding or maternal behaviours.

In summary, maternal sensitivity and bioactive factors in breast milk (i.e. cortisol) are two of the potential mechanisms by which breastfeeding may protect against excessive weight gain in infancy. Whilst previous research has examined relationships between breastfeeding and maternal sensitivity and breastfeeding and cortisol level, reactivity and recovery, there are no studies that have yet considered the importance of the *metabolism* of cortisol rather than cortisol level. Furthermore, there is no longitudinal study published that investigates relationships between all of these variables throughout the first year of life. Undertaking a study of this nature will allow us to investigate in more depth, the links between breastfeeding, maternal behaviour and infant physiology. It is important to establish whether or not relationships exist between these variables before additionally examining these variables among effects of infant weight and eating behaviours.

5.1.1 Aims and hypotheses

The aim of this study was to investigate potential associations between breastfeeding duration, positive maternal mealtime behaviours and cortisol metabolism at 6- and

12-months-of-age. It is hypothesised that a longer duration of breastfeeding will be associated with (1) more positive maternal mealtime interactions; (2) increased cortisol metabolism in infants; and (3) increased cortisol metabolism in mothers. It is also hypothesised that there will be a positive association between positive maternal interactions and infant cortisol metabolism at 6- and 12-months. It is expected that increased cortisol metabolism will be associated with increased breastfeeding duration and more positive maternal interactions as previous research has demonstrated that these maternal behaviours are related to faster cortisol recovery. As persistently elevated cortisol can have negative effects on HPA-axis functioning, faster cortisol recovery and increased cortisol metabolism are considered adaptive and positive.

5.2 Methods

5.2.1 Participants

Eighty-one full-term infants (45 males, 36 females; mean birth-weight 3.52 kg [SD 0.39]), and their mothers (mean age 29.42 years [SD 5.87]) were recruited from low-risk maternity units of the Birmingham Women's Hospital (refer to Chapter 2 for more information on eligibility, recruitment and ethical approval).

Of the 81 mother-infant dyads initially recruited, 58 mothers consented to being observed at 6-months and 55 at 12-months (see results section for tests for demographic differences between those who were and were not observed); 59 mothers and infants provided urine samples for the analysis of cortisol metabolism.

5.2.2 Materials and procedure

Mothers were given information sheets on the maternity unit (Appendix 2 & 4) and consent was obtained for the researcher to telephone them to assess willingness to participate in the study (Appendix 3). Mothers happy to participate in the study were visited at home at approximately 1-week postpartum where consent forms were signed by the researcher and participant (Appendix 5).

Mothers and infants were visited at home when the infant was roughly 1-week, 1-month, 6-months and 12-months old. At each time point of the study mothers reported: feeding information (this included details regarding breast- and formula-feeding exclusivity and duration and when solids were first introduced); smoking and alcohol consumption; and any medications they were taking. Demographics were reported at the 1-week visit and mothers completed a questionnaire assessing symptoms of postnatal depression at 1-, 6- and 12-months.

Urine samples were collected at the 1-week, 1-, 6- and 12-month visits. At 3-months, participants were contacted via telephone and urine samples were collected from those who had agreed to provide them. For those who had provided consent, mothers were also observed feeding their infant at the 6- and 12-month home visits

Measures used in the current study include: Demographic and Additional Information (Appendix 7); Additional Information (Appendix 8); Feeding Information (Appendix 10); Edinburgh Postnatal Depression Scale (EPDS; Cox, Holden & Sagovsky, 1987);

Measurement of Infant Cortisol Metabolism (see Chapter 2, section 2.5 for details regarding each measure). The data in this chapter refer primarily to the 6- and 12-month visits.

Mealtime Observation

The Feeding Interaction Scale (FIS; Wolke et al., 1987) was used to code the observations of the mother-infant feeding interaction at the 6- and 12-month home visits. For the current study, it was used to assess positive maternal behaviours during a mealtime including frequency of verbal involvement, appropriateness and sensitivity (refer to Table 2.2 in Chapter 2 for more information on the subscales used and behaviours assessed). As explained in Chapter 2, maternal vocalisations and appropriateness were chosen in addition to sensitivity to allow the investigation of several observable positive feeding behaviours. The FIS has clinical validity and has been used to assess maternal-infant feeding interactions and diagnose feeding problems (Farrow & Blissett, 2005; Lingberg et al., 1996; Skuse et al., 1992).

Feeding sessions took place at participants' homes and were recorded using a video-camcorder and tripod. Videos were watched and scored later by the researcher and research assistant. Intra-class correlation coefficients were all greater than .76. Mothers decided what food to feed their infant during the feeding session. As meal content can affect interactions during mealtimes, it is important to highlight maternal ratings of their infant's familiarity with and liking of the food they were given during the feeding session. As can be seen in Table 5.1, during the mealtime observations, infants were more likely to be given food they were familiar with and food they liked.

Table 5.1

Maternal ratings of infant's familiarity and liking of food given during feeding sessions

	Familiarity				Liking (Mean and S.D.)
	Never	Once	A few times	Often	
6-months	5.2%	10.3%	32.8%	51.7%	82.4% (S.D. 17.0%)
12-months	3.6%	5.5%	25.5%	65.5%	80.1% (S.D. 17.0%)

5.2.3 Data analysis

Kolmogorov-Smirnov tests and histograms indicated that breastfeeding duration, observed maternal sensitivity, infant steroid ratios and demographic factors were not normally distributed. Two-tailed non-parametric Spearman's rho correlations were used to assess whether demographic variables were associated with breastfeeding. Spearman's rho correlations were also used to establish whether maternal postnatal depression, as measured by the EPDS, was related to maternal mealtime behaviours.

Although breastfeeding and steroid ratios were predominantly non-normally distributed (as indicated by Kolmogorov-Smirnov tests and histograms), some parametric tests were used where it was necessary to investigate the existence of possible relationships between variables whilst controlling for covariates. One-tailed partial correlations (controlling for: household income, maternal age, education, and quantity of cigarettes smoked during pregnancy and infant age introduced to solids)

were used to investigate potential relationships between breastfeeding duration and observed maternal sensitivity during feeding.

Two-tailed partial correlations were also conducted, which in addition to the above covariates, also controlled for infant gender and weight SDS to investigate potential relationships between breastfeeding duration and infant steroid ratios indicative of cortisol metabolism. Lastly, two-tailed partial correlations were conducted, controlling for the aforementioned covariates, to investigate potential relationships between observed maternal sensitivity during feeding and infant steroid ratios indicative of cortisol metabolism.

5.3 Results

5.3.1 Descriptive statistics

Of the 81 mother-infant dyads initially recruited into the study, 8 had withdrawn by the 6-month visit and a total of 12 had withdrawn by the 12-month visit (14.8%). Table 3.1 in Chapter 3, section 3.3.1, shows the number of mother-infant dyads seen at each home visit, the mean age of infants (in weeks) at each home visit and the percentage of infants being breastfed (includes exclusive breastfeeding and infants receiving both breast milk and formula). As described in Chapter 3, there was no difference in breastfeeding duration between male ($M = 24.34$, $SE = 3.58$) and female ($M = 30.58$, $SE = 4.03$) infants $t(67) = -1.16$, $p = .25$.

Of the 73 families visited at 6-months, 58 (79%) mothers agreed to be observed feeding their infant. There were no differences in household income, maternal age or education between those who were and were not observed feeding their infants at 6-months (see Appendix 17).

Of the 69 families visited at 12-months, 55 (80%) were observed during a mealtime. There were no differences in household income or maternal age between mothers who did and did not agree to be observed feeding their infants at 12-months (see Appendix 17). However, mothers who agreed to be observed feeding their infant at 12-months were significantly more educated ($M = 6.62$, $SE = 0.31$) than those who did not agree ($M = 5.07$, $SE = 0.62$) to being observed $t(67) = 2.27$, $p < .05$.

5.3.2 Covariates

As described in Chapter 3, two-tailed Spearman's rho correlations revealed that maternal age and educational level were significantly associated with breastfeeding duration at each time point of the study (see Chapter 3, Table 3.3). There were positive associations between breastfeeding duration and household income at 1-week, 1- and 6-months; positive associations between breastfeeding and age infants were introduced to solid food at 6- and 12-months; and negative associations between breastfeeding and cigarettes smoked during pregnancy at 1- and 6-months (plus a trend for this association at 1-week). The aforementioned variables were controlled for in analyses involving breastfeeding and maternal behaviours. Analyses

involving infant steroid ratios additionally controlled for infant weight SDS due to the significant associations found in Chapter 4.

As described in Chapters 3 and 4, maternal postnatal depression, as measured by the EPDS, was not related to breastfeeding or infant steroid ratios. One-tailed Spearman's rho correlations were then conducted to investigate whether EPDS score was related to observed maternal behaviours. As can be seen from Table 5.2, there were no significant relationships between these variables. Maternal postnatal depression was therefore not entered as a covariate in subsequent analyses.

5.3.3 Breastfeeding and positive maternal interactions

One-tailed partial correlations were conducted to investigate the relationship between breastfeeding duration and observed maternal mealtime behaviours, coded using the FIS. Covariates controlled for include: maternal age, education, number of cigarettes smoked during pregnancy, household income and age infant was introduced to solids. Table 5.3 shows a positive relationship between (concurrent) breastfeeding duration and maternal sensitivity and appropriateness during the mealtime at the 6-month visit. At 12-months, these relationships remain, and become stronger. There is also a significant positive association showing a small effect between breastfeeding duration and maternal vocalisations at 12 months.

Table 5.2

Bivariate correlations (one-tailed) between concurrent EPDS score and observed maternal mealtime behaviours at 6- and 12-months.

	6-months			12-months		
	Vocalisations	Appropriateness	Sensitivity	Vocalisations	Appropriateness	Sensitivity
EPDS score	.17	.06	.12	-.02	.03	-.10
n	56	56	56	55	55	55

Table 5.3

Partial correlations (one-tailed) between concurrent breastfeeding duration and observed maternal mealtime behaviours, controlling for: maternal age, education, number of cigarettes smoked during pregnancy, household income and age infant was introduced to solids.

	6-months			12-months		
	Vocalisations	Appropriateness	Sensitivity	Vocalisations	Appropriateness	Sensitivity
Breastfeeding Duration	.07	.23Δ	.23Δ	.24*	.33*	.30*
df	49	49	49	48	48	48
		$\Delta p = .05$	* $p < .05$	** $p < .01$		

5.3.4 Breastfeeding and cortisol metabolism

Two-tailed partial correlations were conducted to investigate the potential relationship between breastfeeding duration and maternal steroid ratios indicative of cortisol metabolism. Covariates controlled for include: maternal age, BMI, education, number of cigarettes smoked during pregnancy and household income. Table 5.4 shows that there were no significant relationships between breastfeeding duration and maternal steroid ratios indicative of cortisol metabolism at any point during the study.

Table 5.4

Partial correlations (two-tailed) between breastfeeding duration and maternal steroid ratios, controlling for: maternal age, BMI, education, number of cigarettes smoked during pregnancy and household income.

Breastfeeding duration	<i>df</i>	Concurrent F/E (11 β -HSD2)	Concurrent THF+5 α THF/THE (11 β -HSD1)	Concurrent 5 α THF/THF (5 α -reductase)
1-week	38	-.04	-.25	.09
1-month	46	.28	-.06	-.05
3-month	39	.20	.11	-.08
6-month	41	-.04	-.16	-.04
12-month	34	-.01	.01	.20

Two-tailed partial correlations were conducted to investigate the potential relationship between breastfeeding duration and infant steroid ratios indicative of cortisol metabolism. Covariates controlled for include: maternal age, education, number of cigarettes smoked during pregnancy, household income and infant gender, weight SDS and age introduced to solid food. Table 5.5 shows a positive association of

medium effect between (concurrent) breastfeeding duration and infant steroid ratios indicative of increased 5 α -reductase at 6-months. At 12-months there were positive associations showing a medium effect between breastfeeding duration and infant steroid ratios indicative of increased 5 α -reductase and 11 β -HSD1.

Table 5.5

Partial correlations (two-tailed) between concurrent breastfeeding duration and infant steroid ratios, controlling for: maternal age, education, number of cigarettes smoked during pregnancy, household income and infant gender, weight and age introduced to solids.

Breastfeeding duration	<i>df</i>	Concurrent F/E (11 β -HSD2)	Concurrent THF+5 α THF/THE (11 β -HSD1)	Concurrent 5 α THF/THF (5 α -reductase)
1-week	37	-.05	.02	.15
1-month	44	.06	.11	-.02
3-month	45	-.17	.15	.02
6-month	41	-.17	.24	.39**
12-month	39	.00	.46**	.38*

* $p < .05$ ** $p < .01$

5.3.5 Positive maternal interactions and cortisol metabolism

Two-tailed partial correlations were conducted to investigate possible associations between maternal steroid ratios and observed maternal mealtime behaviours. Covariates controlled for include: maternal age, education, number of cigarettes smoked during pregnancy, concurrent breastfeeding duration and household income. Table 5.6 shows a negative association of large effect between maternal sensitivity and maternal steroid ratio F/E at 12-months, suggesting more sensitive mothers clear

more cortisol via 11 β -HSD2 at 12-months. There were also trends for medium negative relationships between maternal ratio F/E and maternal positive vocalisations and appropriateness at 12-months. There were no associations between maternal steroid ratios indicative of 11 β -HSD1 and 5 α -reductase at 12-months and no associations between maternal behaviours and maternal ratios at 6-months.

Table 5.6

Partial correlations (two-tailed) between observed maternal mealtime behaviours and maternal steroid ratios, controlling for: maternal age, education, number of cigarettes smoked during pregnancy, concurrent breastfeeding duration and household income.

		<i>df</i>	Concurrent F/E (11 β -HSD2)	Concurrent THF+5 α THF/THE (11 β -HSD1)	Concurrent 5 α THF/THF (5 α -reductase)
6- months	Vocalisations	35	-.20	.17	.06
	Appropriateness	35	-.19	-.15	-.05
	Sensitivity	35	-.22	-.28	-.01
12- months	Vocalisations	30	-.34†	.19	.15
	Appropriateness	30	-.32†	.02	.14
	Sensitivity	30	-.53**	.07	.14
		† <i>p</i> < 0.08	* <i>p</i> < .05	** <i>p</i> < .01	

Two-tailed partial correlations were then conducted to investigate possible relationships between observed maternal mealtime behaviours and infant steroid ratios indicative of cortisol metabolism. Covariates controlled for include: maternal age, education, number of cigarettes smoked during pregnancy, household income and infant gender, weight SDS and age introduced to solid food. Table 5.7 shows significant positive associations of medium effect between maternal vocalisations and infant steroid ratios indicative of 11 β -HSD1 and 5 α -reductase at 12-months and

maternal appropriateness and infant steroid ratios indicative of 11 β -HSD1 at 12-months. There were no significant associations between infant steroid ratios and maternal sensitivity at 12-months and no associations between maternal behaviours and infant ratios at 6-months.

Table 5.7

Partial correlations (two-tailed) between observed maternal mealtime behaviours and infant steroid ratios, controlling for: maternal age, education, number of cigarettes smoked during pregnancy, household income and infant, gender, weight and age introduced to solids.

		<i>df</i>	Concurrent F/E (11 β -HSD2)	Concurrent THF+5 α THF/THE (11 β -HSD1)	Concurrent 5 α THF/THF (5 α -reductase)
6- months	Vocalisations	34	-.17	-.07	-.02
	Appropriateness	34	-.10	-.01	-.17
	Sensitivity	34	-.19	.15	.02
12- months	Vocalisations	36	-.15	.36*	.38*
	Appropriateness	36	-.24	.33*	.25
	Sensitivity	36	-.24	.19	.16

* $p < .05$ ** $p < .01$

Two-tailed partial correlations were then repeated and in addition to the aforementioned covariates, also included concurrent breastfeeding duration. By controlling for breastfeeding duration, it is possible to investigate whether maternal behaviours are related to the metabolism of cortisol, independently of whether or not mothers breastfeed their infants, and how long they breastfeed them for. Table 5.8 shows a significant positive association of medium effect between maternal vocalisations and steroid ratios indicative of 5 α -reductase at 12-months. There was a trend for a medium effect between maternal vocalisations and ratios indicative of

11 β -HSD1 at 12-months. The relationship previously seen between maternal appropriateness and ratios indicative of 11 β -HSD1 was no longer significant. Furthermore, there were no associations between infant steroid ratios and maternal sensitivity at 12-months and no associations between maternal behaviours and infant ratios at 6-months.

Table 5.8

Partial correlations (two-tailed) between observed maternal mealtime behaviours and infant steroid ratios, controlling for: maternal age, education, number of cigarettes smoked during pregnancy, household income, concurrent breastfeeding duration and infant, gender, weight and age introduced to solids.

		<i>df</i>	Concurrent F/E (11 β -HSD2)	Concurrent THF+5 α THF/THE (11 β -HSD1)	Concurrent 5 α THF/THF (5 α -reductase)
6- months	Vocalisations	33	-.19	-.03	.04
	Appropriateness	33	-.10	-.04	-.22
	Sensitivity	33	-.18	.11	-.04
12- months	Vocalisations	33	-.16	.31†	.33Δ
	Appropriateness	33	-.26	.26	.17
	Sensitivity	33	-.26	.09	.07

† *p* < .08 Δ *p* = .05

5.4 Discussion

This study aimed to investigate potential associations between breastfeeding duration, positive maternal mealtime interactions and cortisol metabolism at 6- and 12-months-of-age. It was hypothesised that a longer duration of breastfeeding would be associated with more positive maternal mealtime interactions and increased cortisol metabolism at 6- and 12-months. It was also hypothesised that there would

be a positive association between maternal mealtime behaviours and infant cortisol metabolism at 6- and 12-months.

Results of the current study show that a longer duration of breastfeeding is significantly associated with greater observed maternal appropriateness and sensitivity during a mealtime at 6-months. At 12-months, these relationships remain and are stronger. At 12-months, increased breastfeeding duration is also associated with increased maternal vocalisations during an observed mealtime. Although causality cannot be inferred due to the correlational nature of these analyses, the observation these relationships become stronger over time suggests it is possible that breastfeeding increases sensitive maternal behaviours. However, it is also true that more sensitive mothers choose to breastfeed, therefore breastfeeding for at least 6-months may be a practice that the most sensitive mothers do. It is also important to acknowledge that the current study does not have any baseline measures of maternal sensitivity.

Previous research has found positive associations between breastfeeding duration and maternal sensitivity at 3-months (Britton et al., 2006) and 14-months postpartum (Tharner et al., 2012). In addition, Gutman et al., (2009) found that mothers who breastfeed demonstrate higher quality interactions with their infants at 12-months. Results of the current study support these previous findings, showing that a longer duration of breastfeeding is associated with more positive maternal interactions during feeding at 6- and 12-months. Furthermore, this study demonstrated these

relationships during a mealtime in participants' own homes rather than in a laboratory setting, such as that used by Tharner et al., (2012).

Results of the current study demonstrate that there appears to be no relationship between breastfeeding duration and maternal steroid ratios indicative of cortisol metabolism. This suggests that whether or not a mother chooses to breastfeed her infant, or for how long, has no association with her own cortisol metabolism. However, results do show that breastfeeding duration is associated with infant steroid ratios. Results suggest that breastfeeding duration is associated with increased cortisol activation (via 11β -HSD1) at 12-months and increased cortisol clearance (via 5α -reductase) at 6- and 12-months, but not at 1-week, 1-, or 3-months.

Spangler et al., (1994) stated that maternal behaviour coordinates infant psychobiological function during the first year of life and earlier research has found that breastfeeding for longer durations predicts quicker cortisol recovery in response to psychological stress (Beijers et al., 2013). The current study did not look at cortisol recovery but it is the first to explore relationships between breastfeeding and cortisol metabolism. The finding that breastfeeding duration is associated with increased cortisol activation and clearance highlights the possibility that breastfeeding for a longer duration may contribute to the programming of the HPA axis during infancy. Future research should seek to clarify whether breastfed infants continue to activate and clear more cortisol, post breastfeeding, in early childhood.

Analyses of maternal mealtime behaviours and maternal steroid ratios indicate that more sensitive mothers clear more cortisol via 11 β -HSD2 at 12-months. There were also trends between greater cortisol clearance by 11 β -HSD2 and maternal positive vocalisations and appropriateness at 12-months. There were no associations between maternal steroid ratios indicative of 11 β -HSD1 and 5 α -reductase at 12-months and no associations between maternal behaviours and maternal steroid ratios at 6-months. It is unclear why positive behaviours appear to be associated with cortisol clearance via 11 β -HSD2, but not 5 α -reductase or cortisol activation. Furthermore, due to the correlational nature of the analyses, it cannot be inferred whether demonstrating more positive behaviours influences cortisol clearance or vice versa. However, raised cortisol for prolonged periods is associated with stress and depression and can have negative effects on HPA-axis functioning, therefore increased clearance of cortisol may be interpreted as adaptive.

Regarding maternal mealtime behaviours and infant steroid ratios, findings of the current study revealed that more maternal vocalisations were associated with increased cortisol activation (via 11 β -HSD1) and clearance (via 5 α -reductase) in infants at 12-months. Also, increased maternal appropriateness during a mealtime was associated with increased cortisol activation (via 11 β -HSD1) at 12-months. Observed maternal sensitivity was not related to infant steroid ratios at 6- or 12-months and there were no relationships between infant steroid ratios and maternal behaviours at 6-months. Given the exploratory nature of these analyses, it is speculated that physiological factors, such as genetics, may programme levels of metabolising enzymes in early infancy. Maternal behaviours may then begin to visibly

influence cortisol metabolism in later infancy. In addition, the absence of a relationship between observed maternal sensitivity and cortisol metabolism in the first year of life does not mean that sensitive maternal behaviours will not have effects on the cortisol metabolism of their offspring later on in life. For example, Murray et al., (2010) investigated parenting behaviour and cortisol secretion in children and discovered that withdrawn parenting is associated with elevated cortisol secretion in offspring at 13-years-old.

Controlling for breastfeeding resulted in the removal of the relationships between infant 11β -HSD1 and maternal vocalisations and appropriateness during a mealtime. The association between infant 5α -reductase activity and maternal vocalisations remained, albeit slightly weakened. These results therefore suggest that breastfeeding may explain some of the relationship between observed maternal behaviours and cortisol activation, via 11β -HSD1, in infants. Furthermore, even when analyses control for breastfeeding, there is an element of the relationship between cortisol clearance, via 5α -reductase, and maternal behaviours that cannot be explained by breastfeeding alone. Figure 5.1 illustrates the significant relationships between variables at 12-months.

Results of the current study are the first to show that positive maternal vocalisations during a mealtime are associated with increased cortisol clearance in 12-month-old infants, even when breastfeeding duration is controlled for. As persistently elevated levels of cortisol can have negative effects on HPA-axis functioning, increased cortisol clearance may be interpreted as adaptive. Although causality cannot be

inferred from these correlational analyses, it is possible that positive maternal behaviours impact on the cortisol metabolism of their offspring in later infancy and beyond. On the other hand, an infant's cortisol metabolism may influence their appetite and how easy they are to feed. An infant who is difficult to feed may then prompt mothers to use less appropriate and positive behaviours during feeding, which is likely to be stressful for mother and child. Therefore, regardless of causality, findings imply that interventions which aim to train parents to interact more positively with their infants may promote healthy cortisol metabolism and HPA-axis function in children.

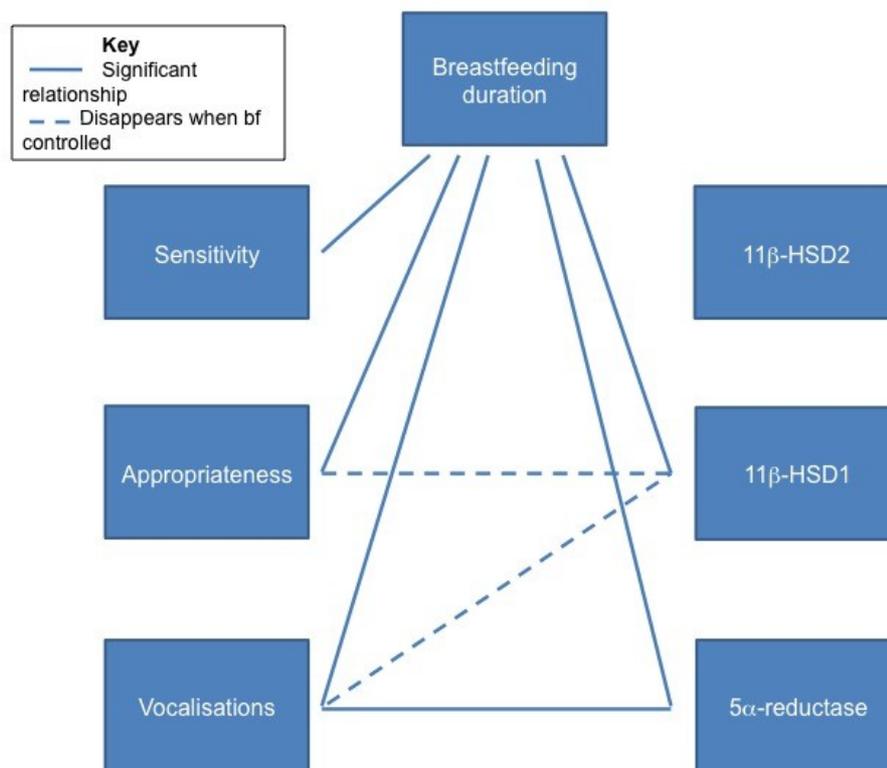


Figure 5.1

Model illustrating the significant relationships between breastfeeding duration, infant cortisol metabolism and maternal behaviours during feeding at 12-months.

One limitation of the current study is that breast milk samples were not collected. Requesting such samples from mothers would have enabled us to examine the content of breast milk and how it varies throughout the day and from mother-to-mother. It would also have allowed us to test the milk for the presence of the enzymes (11β -HSD1, 11β -HSD2 and 5α -reductase) involved in cortisol metabolism and from this it would be clear how much of each enzyme infants are directly exposed to through breast milk. Also, as mentioned in Chapter 4, it cannot be guaranteed that all nappy samples were 24-hour collections. This is because mothers were requested to dispose of soiled nappies and may have forgotten to start collecting at the correct time. Therefore some nappies will invariably have been 'missed'. To prevent this being too much of an issue, participants were reminded to start collecting nappies the day before the visit took place by the researcher.

Another limitation of the current study is that exclusivity of breastfeeding was not accounted for in analyses, only duration. It is possible that if breastfeeding exclusivity was assessed in addition to duration, stronger relationships with maternal sensitivity and cortisol metabolism may have been observed. Furthermore, a higher proportion of women in the current study had been through higher education and a higher proportion was breastfeeding at 6-months compared to the UK average (52% versus 34% [McAndrew et al., 2010a]). Given the results of this study, and those prior to it, this may mean that the current sample consisted of a larger number of more sensitive mothers. Future research should seek to recruit a sample more representative of the UK population.

Limitations considered, it is important to highlight this study's strengths. Firstly, maternal behaviours were observed through naturalistic observations of a mealtime at 6- and 12-months. Mothers were observed feeding their infants in their own homes and the researcher did not sit in the same room wherever possible. This is a more objective measure than relying on maternal self-report and ensured the observation of more representative mealtimes and behaviours. In addition to this, due to the longitudinal nature of the study, by the time participants were observed they had already met with the researcher at least three times and spoken to them significantly more. This meant that a good rapport between the researcher and participants had already been established and so participants were happier to be observed and were more relaxed during recording. Further to this, this is the first study to have investigated maternal behaviour and cortisol metabolism rather than cortisol level, reactivity or recovery. Investigating the metabolism rather than level of cortisol gives a global measure of the proportion of active and inactive glucocorticoid from liver fat and muscle.

Given the relationships between breastfeeding and weight gain (Chapter 3), breastfeeding and cortisol metabolism (the current study), and cortisol metabolism and weight (Chapter 4) in infancy, the next chapter will explore the interaction of these factors incorporating maternal sensitivity and infant eating behaviours in one study. Investigation of these variables may help to increase understanding of the early contributors to excessive weight gain, obesity and obesogenic eating behaviours. This increased understanding is required in order to develop effective preventions and interventions of obesity.

In conclusion, this study is the first to have investigated the relationships between breastfeeding, maternal sensitivity and infant cortisol metabolism longitudinally over the first postnatal year. Findings suggest that maternal appropriateness and positive vocalisations during a mealtime are associated with infant steroid ratios indicative of increased cortisol activation and clearance at 12-months, and that a longer duration of breastfeeding is associated with increased cortisol activation at 12-months and increased cortisol clearance at 6- and 12-months. When relationships between maternal interactions and steroid ratios are examined controlling for breastfeeding duration, maternal appropriateness appears to no longer be related to cortisol activation; however, even when breastfeeding duration is controlled for, mothers who speak more positively and frequently to their infants during feeding, have babies who clear significantly more cortisol. Future research should seek to clarify whether breastfeeding's effect on cortisol metabolism and maternal behaviours persists into early childhood, post-breastfeeding.

CHAPTER 6

PREDICTORS OF INFANT EATING BEHAVIOURS AND WEIGHT GAIN

Abstract

Background: Results of Chapter 4 suggest that reduced clearance of cortisol (via 11 β -HSD2) is associated with slower weight gain throughout the first year of life. However, possible relationships between infant cortisol metabolism and eating behaviours have not yet been investigated. The current study aimed to explore the key variables presented within this thesis (including breastfeeding duration, positive maternal mealtime behaviours and steroid ratios indicative of cortisol metabolism) that may predict infant eating behaviours at 12-months and weight gain over the first year of life.

Method: Eighty-one mothers and their infants were recruited from low-risk maternity units; 69 of these were visited at home at 12-months postpartum and provided questionnaire measures of infant eating behaviour. Forty-eight mothers provided 24-hour nappy collections from infants for the analysis of cortisol metabolism and 55 were observed feeding their infant. Observations were coded for infant food acceptance, maternal vocalisations and appropriateness and sensitivity of behaviour during the mealtime.

Results: Partial correlations revealed positive associations between total infant food acceptances and maternal positive interactions during an observed mealtime. Partial correlations also revealed that lower infant 11 β -HSD2 activity was associated with increased food acceptances during a meal and increased infant 5 α -reductase activity

was associated with slower eating, as perceived by mothers. Regression analyses found that infant enjoyment of food was predicted by age of introduction to solid food and infant slowness in eating was predicted by more cortisol clearance, via increased 5α -reductase. Total observed food acceptances during a meal was predicted by less cortisol clearance, via 11β -HSD2. Slower infant weight gain was predicted by an increased duration of breastfeeding, lower level of maternal education, lower enjoyment of food and food fussiness (as perceived by the mother) and reduced clearance of cortisol via reduced 11β -HSD2 activity.

Discussion: Infant cortisol metabolism is a primary predictor of infant eating behaviours, over and above other known predictors including breastfeeding, weight gain and maternal sensitivity. Slower patterns of weight gain are predicted by a longer duration of breastfeeding, less food enjoyment and fussiness and reduced cortisol clearance (via 11β -HSD2). Future research should investigate whether these relationships persist into early childhood.

6.1 Introduction

Maternal behaviour during feeding has an effect on the development of their offspring's eating behaviours and weight gain later in life. For example, by exposing infants to particular foods and through modelling certain behaviours (Wardle et al., 2005), parents influence the eating behaviours of their infants and children. In addition to this, and as discussed in Chapter 1 (section 1.2.4), how accurately mothers perceive and interpret their infant's signals, and how prompt and appropriate their responses are, have important implications for later eating behaviours and

weight gain. Mothers who miss or ignore their infant's signals, or who do not respond to their infant quickly or appropriately, may override their infants internal hunger and satiety cues and affect the development of eating behaviours. For example, Worobey et al., (2009) found that less sensitive mothers are more likely to overfeed their infants.

Maternal behaviours during feeding that are more restrictive and less sensitive have been associated with increased snacking behaviour (Birch & Fisher, 2000; Fisher & Birch, 1999) and higher body fat in children (Spruijt-Metz et al., 2002) and may therefore increase children's risk of overweight (Davison & Birch, 2001). This thesis has not yet investigated potential relationships between positive maternal interactions during feeding and infant eating behaviours. The current chapter will do so and if relationships exist, further analysis will involve testing whether such eating behaviours and weight gain can be predicted by observed maternal behaviours during a mealtime.

Maternal sensitivity is also linked to breastfeeding; more sensitive mothers choose to breastfeed, and also breastfeed for longer durations (Britton et al., 2006). In support of this research, results of Chapter 5 have demonstrated that duration of breastfeeding is related to positive maternal mealtime interactions, including vocalisations, appropriateness and sensitivity (refer to Figure 6.1 below, page 176) for a diagrammatic representation of significant relationships found in Chapters 3, 4 and 5).

Breastfeeding itself is associated with slower weight gain from 3- to 6- and 6- to 9-months (Heinig et al., 1993) and positive eating behaviours, such as higher fruit and vegetable consumption (Kudlová & Schneidrová, 2012) and increased satiety responsiveness (Brown & Lee, 2012). In support of these previous findings, results of Chapter 3 show that a longer duration of breastfeeding is associated with slower weight gain from 1- to 12-months and increased maternal report of slower eating styles.

In addition to maternal behaviours and breastfeeding, an infant's eating behaviour and weight gain may also be influenced by their cortisol metabolism. As discussed in Chapter 1, cortisol has been related to obesogenic eating behaviours and overweight in human adults (Björntorp & Rosmond, 2000; Dallman et al., 2003; Marin et al., 1992; Pasquali et al., 1993; Tataranni et al., 1996). Dallman et al., (2003) reviewed previous research and found that increased glucocorticoids in rats (that occur when they are stressed) induce the consumption of comfort foods higher in sucrose and fat. The authors also argue that this effect can also be seen in humans.

As discussed throughout this thesis, it is now suggested that investigating cortisol metabolism, rather than cortisol level, may have more important implications for weight gain. For example, obese humans usually have normal circulating cortisol levels, but their rates of cortisol clearance and production are often increased (Andrew et al., 1988; Björntorp & Rosmond, 2000; Vierhapper et al., 2004). Results in Chapter 4 are the first to suggest that reduced clearance of cortisol (via the enzyme, 11 β -HSD2) is associated with slower weight gain through the first year of

life (see Figure 6.1, page 176). Analyses in this thesis have not yet explored the possible relationships between infant cortisol metabolism and eating behaviours. The current chapter will examine these relationships and use regression analyses to establish the best predictors of weight gain and eating behaviours at 12-months; doing so will identify the relative importance of the predictors explored throughout the thesis in their prediction of eating behaviours and weight gain in infancy.

6.1.1 Aims and Hypotheses

The first aim of this study was to investigate potential associations between positive maternal mealtime behaviours, cortisol metabolism and infant eating behaviours at 12-months-of-age. It was hypothesised that more positive maternal mealtime behaviours will be associated with more positive observed and maternally reported eating behaviours. It was also hypothesised that infant eating behaviours, both observed and maternally reported, will be associated with infant cortisol metabolism.

This study also aimed to explore the variables that may predict infant eating behaviours at 12-months and weight gain over the first year of life. Due to the range of variables that have been considered thus far, this chapter took a pragmatic approach to deciding which key variables to include in the final analyses. Infant eating behaviours significantly related to variables of interest in this thesis were chosen to be predicted. Based on the results of previous chapters of the current thesis (see Figure 6.1), it is hypothesised that breastfeeding duration, positive

maternal mealtime behaviours and steroid ratios indicative of cortisol metabolism will all be significant predictors of infant weight gain and eating behaviours at 12-months.

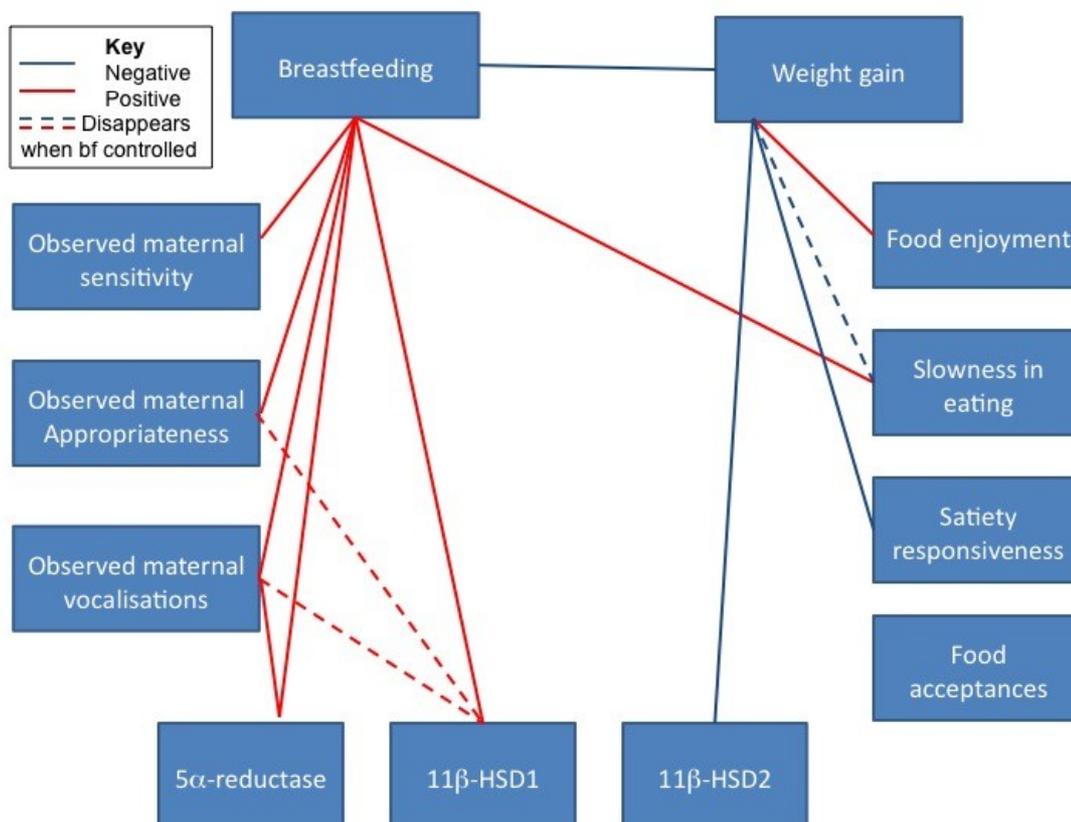


Figure 6.1

Significant relationships between breastfeeding duration, weight gain, infant eating behaviours, cortisol metabolism and positive maternal mealtime behaviours.

6.2 Methods

6.2.1 Participants

Eighty-one full-term infants (45 males, 36 females; mean birth-weight 3.52 kg [SD 0.39]), and their mothers (mean age 29.42 years [SD 5.87]) were recruited from low-

risk maternity units of the Birmingham Women's Hospital (refer to Chapter 2 for more information on eligibility, recruitment and ethical approval).

Of the 81 mother-infant dyads initially recruited, 55 mothers consented to being observed at 12-months (see Chapter 5, section 5.3.1 for tests of demographic differences between those who were and were not observed); 59 mothers provided 24-hour nappy collections for the analysis of infant cortisol metabolism (see Chapter 4, section 4.3.1 for tests of demographic differences between mothers who did, and did not, agree to provide samples).

6.2.2 Materials and procedure

As previously stated, mothers and their new-born infants were approached on low risk maternity units of Birmingham Women's Hospital (please refer to Chapter 2, sections 2.1 and 2.2, for details). Mothers happy to participate in the study were visited at home at approximately 1-week, 1-month, 6- and 12-months postpartum. Mothers and infants were weighed and measured by the researcher at each visit.

At the 12-month visit, mothers reported: feeding information (this included details regarding breast- and formula-feeding exclusivity and duration and when solids were first introduced) and completed a questionnaire assessing their infant's eating behaviours. Demographics were reported at the 1-week visit. Mothers who had provided nappy collections at previous study time points also did so at the 12-month

visit. For those who had provided consent, mothers were also observed and recorded feeding their infant.

Measures used in the current analysis include: Demographic and Additional Information (Appendix 7); Additional Information (Appendix 8); Feeding Information (Appendix 10); Child Eating Behaviour Questionnaire (CEBQ; Wardle et al., 2001); total observed food acceptances, positive maternal interactions and Measurement of Infant Cortisol Metabolism (see Chapter 2, section 2.5 for details regarding each measure). The data in this chapter refers primarily to the 12-month visit.

Mealtime Observation

Feeding sessions took place at participants' homes and were recorded using a video-camcorder and tripod. Videos were watched and scored later by the researcher and research assistant. Intraclass correlation coefficients were all greater than .76 (see Appendix 19). Recordings were used to establish the total number of infant food acceptances that occurred during the mealtime; this measure of total food acceptances served as an index of appetite and ease of feeding.

The Feeding Interaction Scale (FIS; Wolke et al., 1987) was also used to code the observations of the mother-infant feeding interaction at the 12-month home visit. As explained in Chapter 5, the FIS was used to assess positive maternal interactions with the infant during a mealtime; interactions analysed included: frequency of positive verbal involvement, appropriateness and sensitivity (refer to Chapter 2,

section 2.5.2, for more information on the clinical validity of the measure and the behaviours assessed).

6.2.3 Data analysis

Although Kolmogorov-Smirnov tests and histograms indicated that observed maternal sensitivity and infant steroid ratios were predominantly non-normally distributed, observed maternal positive vocalisations and appropriateness and infant eating behaviours were normally distributed. Parametric tests were therefore used where it was necessary to investigate the existence of possible relationships between variables whilst controlling for covariates.

Firstly, one-tailed partial correlations (controlling for: household income, breastfeeding duration, maternal age, education, infant weight SDS and age introduced to solid food) were used to investigate potential relationships between positive maternal mealtime interactions and infant food acceptances and eating behaviours, as measured by the CEBQ.

Secondly, two-tailed partial correlations (controlling for: household income, maternal age, education and BMI, infant gender, weight SDS and age introduced to solid food) were conducted to investigate the potential relationships between infant steroid ratios and infant food acceptances and eating behaviours, as measured by the CEBQ. These analyses were also run again controlling for breastfeeding duration to

establish whether breastfeeding could explain part of any relationship between infant cortisol metabolism and eating behaviours.

Finally, regression analyses were performed in order to predict infant weight gain and eating behaviours. To decide which key variables to predict, a pragmatic approach was utilised whereby only the infant eating behaviours that were significantly related to variables of interest in Chapter 3 and the current chapter were chosen to be predicted (maternal reports of satiety responsiveness, slowness in eating, enjoyment of food, food fussiness and observations of total infant food acceptances). Predictors entered into regression analyses were the variables and demographic factors significantly related to eating behaviours and weight throughout the thesis. Due to the fact that these were exploratory analyses in which was unclear which of the variables would be the primary predictors, stepwise regressions were used whereby the order in which predictors are entered into the model is based on mathematical criterion. The predictor variable that best predicts the outcome variable is selected and if it significantly improves the model's ability to predict the outcome, it is retained in the model. Should this occur, a second predictor is searched for, and so on.

6.3 Results

6.3.1 Descriptive statistics

Of the 81 mother-infant dyads initially recruited into the study, 69 of them participated at the 12-month follow-up visit. At 12-months, 32% of mothers were still breastfeeding and 64% of these had not introduced formula or cow's milk. There were no male infants below the 2nd and there were no female infants below the 9th

centile for weight at 1-, 6- or 12-months (according to UK-WHO growth charts [Appendix 1]); this suggests that there were no significantly underweight infants in the current sample (see Chapter 3, section 3.3.1).

Of the 59 mothers who initially agreed to provide urine samples, 48 provided nappy collections for the analysis of infant cortisol metabolism at the 12-month visit (see Chapter 4, section 4.3.1, for tests of demographic differences between those who did and did not agree to provide urine samples during the study).

As described in Chapter 5, 55 of the 69 mother-infant dyads visited at 12-months were observed during a mealtime (see section 5.3.1 for demographic differences between those who were, and were not, observed feeding their infant).

6.3.2 Infant eating behaviours and observed maternal behaviours

One-tailed partial correlations were performed to investigate the relationship between positive maternal mealtime interactions and infant food acceptances and eating behaviours, as measured by the CEBQ. Variables controlled for include: household income, breastfeeding duration, maternal age, education, infant weight and age introduced to solid food.

Table 6.1 shows significant negative relationships, of large effect, between total infant food acceptances during a meal and maternal positive vocalisations, appropriateness and sensitivity. There is also a significant negative association,

showing a small effect, between maternal report of infant food fussiness and observed maternal appropriateness during a mealtime.

Table 6.1

Partial correlations (one-tailed) between observed maternal mealtime behaviours and infant eating behaviours, controlling for: household income, breastfeeding duration, maternal age, education, infant weight and age introduced to solid food.

	Total acceptances	Satiety responsiveness	Enjoyment of food	Food responsiveness	Slowness in eating	Food fussiness	Desire to drink
<i>df</i>	12	47	47	47	47	47	47
Vocalisations	-.56*	.00	.02	-.07	.13	-.01	.08
Appropriateness	-.59*	-.07	.01	.05	.12	-.23Δ	.08
Sensitivity	-.48*	-.07	.02	-.03	.11	-.08	.08

$\Delta p = .05$ $*p < .05$

6.3.3 Infant eating behaviours and cortisol metabolism

Two-tailed partial correlations were conducted to investigate the potential relationships between infant steroid ratios, observed food acceptances and eating behaviours, as measured by the CEBQ. Covariates controlled for include: household income, maternal age, education and BMI, infant gender, weight SDS and age introduced to solid food. Table 6.2 shows a positive association, of medium effect, between steroid ratio F/E and total infant food acceptances during a meal. This suggests that lower 11β -HSD2 activity is significantly associated with more food acceptances during a meal. Table 6.2 also shows a positive relationship, of medium effect, between steroid ratio 5α THF/THF (indicative of 5α -reductase) and slowness in eating, as measured by the CEBQ. When controlling for breastfeeding duration in addition to the aforementioned covariates, the same relationships remain, of similar strength (see Table 6.3). Figure 6.2 illustrates the significant relationships from the partial correlations in sections 6.3.2 and 6.3.3.

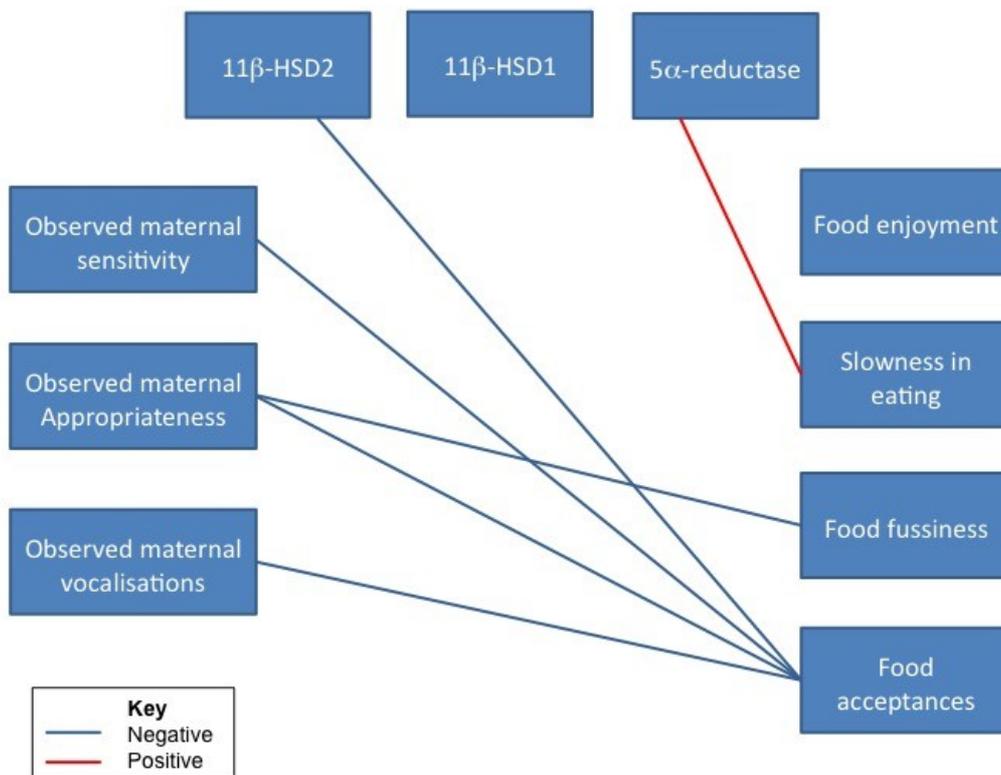


Figure 6.2

Significant relationships between infant eating behaviours, cortisol metabolism and positive maternal mealtime behaviours.

Table 6.2

Partial correlations (two-tailed) between infant steroid ratios and infant eating behaviours, controlling for: household income, maternal age, education and BMI, infant gender, weight SDS and age introduced to solid food.

	Total acceptances	Satiety responsiveness	Enjoyment of food	Food responsiveness	Slowness in eating	Food fussiness	Desire to drink
<i>df</i>	34	39	39	39	39	39	39
F/E (11 β -HSD2)	.32Δ	.10	.09	-.19	.18	-.05	-.01
THF+5 α THF/THE (11 β -HSD1)	.15	-.09	.21	.04	.19	-.13	-.06
5 α THF/THF (5 α -reductase)	.03	.02	.20	-.15	.39*	-.00	.05
		$\Delta p = .05$	$* p < .05$				

Table 6.3

Partial correlations (two-tailed) between infant steroid ratios and infant eating behaviours, controlling for: household income, concurrent breastfeeding duration, maternal age, education and BMI, infant gender, weight SDS and age introduced to solid food.

	Total acceptances	Satiety responsiveness	Enjoyment of food	Food responsiveness	Slowness in eating	Food fussiness	Desire to drink
<i>df</i>	33	38	38	38	38	38	38
F/E (11 β -HSD2)	.33Δ	.10	.09	-.19	.18	-.05	-.01
THF+5 α THF/THE (11 β -HSD1)	.21	-.10	.22	.06	.13	-.14	-.03
5 α THF/THF (5 α -reductase)	.06	.02	.20	-.15	.36*	.00	.09
		$\Delta p = .05$	$* p < .05$				

6.3.4 Predictors of eating behaviours and weight gain

Six individual stepwise regressions were performed to establish the significant predictors of satiety responsiveness, slowness in eating, enjoyment of food, food fussiness, total observed food acceptances during a meal and 1- to 12-month weight gain SDS. The following variables were entered into each of the regressions: household income, maternal age, education, BMI, and positive maternal mealtime behaviours (vocalisations, appropriateness and sensitivity), breastfeeding duration, infant gender, and infant steroid ratios indicative of 11 β -HSD1, 11 β -HSD2 and 5 α -reductase. 1- to 12-month weight gain SDS was also entered into all analyses except for that predicting infant weight gain.

The first and second stepwise regression analyses did not reveal any significant predictors of satiety responsiveness or food fussiness, as measured by the CEBQ.

The third stepwise regression found that the age of the infant when introduced to solid food was a significant predictor of maternal reports of infant enjoyment of food, as measured by the CEBQ, $R^2 = .17$, $F(1, 41) = 8.64$, $p < .01$. The age of the infant when they were introduced to solid food contributed individually and significantly to the prediction of their enjoyment of food (see Table 6.4). The model therefore predicts that, as the age of the infant when introduced to solid food increases by one standard deviation (3.65 weeks), maternal perception of infant food enjoyment decreases by .42 standard deviations. The standard deviation for enjoyment of food is 0.68 and so this constitutes a change of 0.29 points. Therefore, if the age of the

infant when introduced to solid food increases by 3.65 weeks, maternal perception of infant food enjoyment will decrease by 0.29 points.

Table 6.4

Stepwise regression predicting enjoyment of food, as measured by the CEBQ.

	<i>B</i>	<i>SE B</i>	β
Constant	5.87	0.55	
Age introduced to solid food	-0.08	0.03	-.42**

** $p < .01$

The fourth stepwise regression found that the infant steroid ratio indicative of 5α -reductase activity was a significant predictor of their slowness in eating, as measured by the CEBQ, $R^2 = .17$, $F(1, 41) = 8.37$, $p < .01$. Steroid ratios indicative of 5α -reductase activity contributed individually and significantly to the prediction of their slowness in eating (see Table 6.5). The model therefore predicts that as ratios indicative of infant 5α -reductase activity increase by one standard deviation (1.02 units), maternal perception of infant slowness in eating increases by .41 standard deviations. The standard deviation for slowness in eating is 0.78 and so this constitutes a change of 0.32 points. Therefore, if ratios indicative of infant 5α -reductase activity increase by 1.02 units, maternal perception of infant slowness in eating will increase by 0.32 points.

Table 6.5

Stepwise regression predicting slowness in eating, as measured by the CEBQ.

	<i>B</i>	<i>SE B</i>	β
Constant	1.48	0.33	
5 α THF/THF (5 α -reductase)	0.31	0.11	.41**

** $p < .01$

The fifth stepwise regression found that the infant steroid ratio F/E (indicative of 11 β -HSD2 activity) was a significant predictor of their observed food consumed, $R^2 = .10$, $F(1, 41) = 4.47$, $p < .05$. Steroid ratios indicative of 11 β -HSD2 activity contributed individually and significantly to the prediction of their observed food consumed (see Table 6.6). An increasing F/E ratio value is indicative of decreasing 11 β -HSD2 activity, the model therefore predicts that as infant 11 β -HSD2 activity decreases by one standard deviation (0.35 units), the total number of observed food acceptances during a meal increases by .31 standard deviations. The standard deviation for total observed food acceptances is 17.36 and so this constitutes a change of 5.38 acceptances. Therefore, if the infant 11 β -HSD2 activity decreases by 0.35 units, the total number of observed food acceptances during a meal will increase by 5.38 acceptances.

Table 6.6

Stepwise regression predicting total observed food acceptances during a meal.

	<i>B</i>	<i>SE B</i>	β
Constant	26.08	6.42	
F/E (11 β -HSD2)	15.73	7.44	.31*

* $p < .05$

The sixth stepwise regression found five significant predictors of infant 1- to 12-month weight gain, $R^2 = .51$, $F(1, 37) = 4.80$, $p < .05$. As can be seen in Table 6.7, breastfeeding duration, maternal education, enjoyment of food, food fussiness and infant steroid ratios indicative of 11 β -HSD2 activity all contributed significantly to the prediction of infant 1- to 12-month weight gain SDS. The model therefore predicts the following:

1.) As breastfeeding duration increases by one standard deviation (147.73 days), weight gain SDS reduces by .35 standard deviations. The standard deviation for 1- to 12-month weight gain SDS is 1.26 and so this constitutes a change of 0.44 points. Therefore, if breastfeeding duration increases by 147.73 days, 1- to 12-month weight gain SDS will be 0.44 points lower.

2.) As maternal education decreases by one standard deviation (2.32 units), weight gain SDS reduces by .40 standard deviations. The standard deviation for 1- to 12-month weight gain SDS is 1.26 and so this constitutes a change of 0.50 points. Therefore, if maternal education decreases by 2.32 units, 1- to 12-month weight gain SDS will be 0.50 points lower. An example of a two-unit difference in maternal

education is one mother having completed a professional qualification without a degree and another mother having completed a further degree.

3.) As maternal perception of infant food enjoyment decreases by one standard deviation (0.68 points), weight gain reduces by .63 standard deviations, which constitutes a change of 0.79 points. Therefore, if maternal perception of infant food enjoyment decreases by 0.68 points, 1- to 12-month weight gain SDS will be 0.79 points lower.

4.) As maternal perception of infant food fussiness decreases by one standard deviation (0.76 points), weight gain reduces by .48 standard deviations, which constitutes a change of 0.60 points. Therefore, if maternal perception of infant food fussiness decreases by 0.76 points, 1- to 12-month weight gain SDS will be 0.60 points lower.

5.) As infant ratio F/E increases (indicating a decrease in 11 β -HSD2 activity) by one standard deviation (0.35 units), weight gain reduces by .26 standard deviations, which constitutes a change of 0.33 points. Therefore, if the infant 11 β -HSD2 activity decreases by 0.35 units, 1- to 12-month weight gain SDS will be 0.33 points lower.

Table 6.7

Stepwise regression predicting 1- to 12-month weight gain SDS.

		<i>B</i>	<i>SE B</i>	β
Model 1	Constant	0.78	0.31	
	Breastfeeding	-0.004	0.001	-.44**
Model 2	Constant	-0.34	0.54	
	Breastfeeding	-0.004	0.001	-.46***
	Maternal education	0.17	0.07	.32*
Model 3	Constant	-2.49	1.14	
	Breastfeeding	-0.004	0.001	-.46***
	Maternal education	0.17	0.07	.32*
	Enjoyment of food	0.51	0.24	.27*
Model 4	Constant	-6.58	2.02	
	Breastfeeding	-0.003	0.001	-.38**
	Maternal education	0.19	0.07	.35**
	Enjoyment of food	1.05	0.32	.56**
	Food fussiness	0.69	0.29	.42*
Model 5	Constant	-6.78	1.93	
	Breastfeeding	-0.003	0.001	-.35**
	Maternal education	0.21	0.06	.40**
	Enjoyment of food	1.17	0.31	.63***
	Food fussiness	0.79	0.28	.48**
	Ratio F/E (11 β -HSD2)	-0.95	0.43	-.26*

p* < .05 *p* < .01 ****p* = .001

6.4 Discussion

The current study aimed to investigate potential associations between positive maternal mealtime behaviours, cortisol metabolism and infant eating behaviours at 12-months-of-age. It was hypothesised that more positive maternal mealtime

behaviours would be associated with positive (observed and maternally reported) infant eating behaviours. It was also hypothesised that infant eating behaviours, both observed and maternally reported, would be associated with infant cortisol metabolism.

This study also aimed to explore the variables that may predict infant eating behaviours at 12-months and weight gain from 1- to 12-months. Based on the results of previous chapters of the current thesis, it was hypothesised that breastfeeding duration, positive maternal mealtime behaviours and steroid ratios indicative of cortisol metabolism would all be significant predictors of infant eating behaviours and weight gain during the first year of life.

Results of the current study demonstrate that the total number of food acceptances during a meal is significantly related to observation of fewer maternal positive vocalisations and less appropriateness and sensitivity during a meal. In addition to this, mothers who perceived their infants to be fussy with food tended to demonstrate less appropriate mealtime behaviour. Mothers observed to be less appropriate during mealtimes may therefore have infants who either under- or overeat. Results also suggest that a greater degree of positive maternal interaction during a meal is associated with less food acceptance and less fussiness, behaviours that may be interpreted as obesity-protective and less problematic during feeding. However, it is also important to consider if a well-liked food was offered during the meal, mothers may not have used vocalisations or other forms of encouragement as those behaviours would not have been required to prompt food consumption.

Previous research by Worobey et al., (2009) found that mothers who regularly overfed their infants were less sensitive to their infant's cues. In addition, Roberts (1991) found that infants who become overweight consume 42% more energy at 6-months than infants who remain lean. Results are supportive of these previous findings as they indicate that mothers who display more positive interactions during feeding may be less likely to overfeed their infants. Furthermore, although Worobey et al., (2009) used observational methods to assess maternal sensitivity, overfeeding was analysed through maternal self-report; the current study however, utilised a naturalistic mealtime observation in participants' own homes to assess maternal feeding behaviours and infant food acceptance. It is important to recognise here that the current study included a measure of food acceptance, not overfeeding. It may therefore be the case that rather than being overfed, these infants may just be eating more during a meal.

Previous research has also found that mothers who are observed to be less sensitive and more controlling during feeding report either having infants with more feeding problems or greater mealtime negativity (Farrow, 2005). Mothers of infants with more feeding problems were also observed to vocalise less during a meal. Furthermore, mothers who reported greater mealtime negativity were observed to be more inappropriate during a meal and their infants accepted less food (Farrow, 2005). Results of the current study support these findings by showing that mothers that are observed to be less appropriate during a meal, report having infants who are fussier with food. Findings of the current study therefore support the notion that mothers who

display more positive interactions during a meal have infants who develop obesity-protective and less problematic eating behaviours.

Due to the correlational nature of the analyses however, it cannot be inferred whether less appropriate maternal behaviour causes infants to be fussy with food, or whether infant fussiness prompts mothers to use increasingly inappropriate behaviour during mealtimes. Interestingly, Powell, Farrow and Meyer (2011) found that maternal report of using more pressuring feeding practices predicted children's food avoidance behaviours. The authors suggest that this relationship is likely to be bi-directional – parents of food avoidant children may pressure them to eat more during a meal, and pressuring feeding practices may cause or exacerbate the development of feeding problems (Powell et al., 2011). Further research is therefore required in order to develop interventions that will encourage positive mealtime interactions between parents and children and prevent problematic eating behaviours developing.

Results of the current study also revealed that infants who have steroid ratios indicative of lower 11β -HSD2 activity, and therefore appear to clear less cortisol via 11β -HSD2, were observed to accept more food during a mealtime. Furthermore, infants who appear to clear more cortisol, via 5α -reductase, are perceived by their mothers to eat slower. Interestingly, breastfeeding duration did not have an effect on these relationships. This suggests that regardless of whether or not mothers breastfeed, or the duration of that feeding, infants who clear less cortisol, via 11β -HSD2, appear to accept more food than those who have steroid ratios indicative of greater cortisol clearance; infants who clear more cortisol, via 5α -reductase, eat

more slowly than those who have steroid ratios indicative of less cortisol clearance. Although it is a little early to speculate about specific mechanisms by which these effects may occur, results suggest that infant cortisol metabolism may play a significant role in infant food acceptance and/or appetite.

Furthermore, regression analyses demonstrated that slowness in eating was predicted by increased cortisol clearance via 5α -reductase, and greater observed food acceptances during a meal was predicted by less cortisol clearance, via 11β -HSD2. The results suggest that cortisol metabolism is a key predictor of these infant eating behaviours, over and above other key variables of interest, including: weight gain, breastfeeding, maternal positive mealtime behaviours and demographics.

The aforementioned findings appear consistent with the literature investigating effects of cortisol level and reactivity on eating behaviours in human adults. Epel et al., (2001) found that women who showed greater cortisol increases (in response to stress) consumed more calories than women who showed smaller cortisol increases. Findings of the current study are consistent in that, increased exposure to cortisol, through limited clearance of it, is associated with increased food acceptances during a meal. However, it is also important to highlight here, that the prediction of total food acceptances by lower cortisol clearance, only reveals that infants ate what they were offered, not necessarily that they ate a lot, or were overeating.

However, the results are not completely consistent with the idea that increased exposure to cortisol is obesogenic, when weight gain is also considered. Results in

Chapter 4 showed that reduced clearance of cortisol (via 11β -HSD2) is associated with *slower* weight gain throughout the first year of life. It is not immediately clear why infants who clear less cortisol (via 11β -HSD2) gain weight more slowly; yet accept more food during a meal. Although, it is possible that infants who eat more during a meal may snack less between meals, thus preventing excessive weight gain, and that there are differences in the relationships between cortisol metabolism, weight and eating, between healthy weight and overweight infants. Within our sample, we do not have extremes of weight at either end of the spectrum and insufficient power to compare weight categories within the sample. It is possible that examination of relationships between cortisol metabolism, weight and eating behaviour in samples of underweight and overweight infants may help to unravel the complexities of these relationships.

It is important to consider here however, that previous research has found that: increased cortisol in response to stress is associated with increased eating in the absence of hunger in 8- to 9-year-olds, but not 5- to 7-year-olds (Francis et al., 2013); and increased snacking in response to daily hassles in adult women (Newman et al., 2007). Therefore, although limited clearance of cortisol may be associated with consuming more during a meal in infancy, and potentially the pre-school years, increased and chronic exposure to cortisol in response to stress in later childhood and the adult years may reduce sensitivity to satiety signals, and as suggested by Adam and Epel (2007), may affect the reward system leading to increased consumption of highly palatable food. The combination of prolonged exposure to

cortisol through stress and increased consumption of dense calories will contribute to fat distribution and weight gain (see Figure 1.2, page 33).

There is currently no published research investigating the metabolism of cortisol and its potential relationships with eating behaviours in either human or animal models. The current study is the first to demonstrate that cortisol metabolism is a key predictor of human eating behaviours in the first year of life. Further research should seek to clarify these initial findings and also investigate whether these relationships persist into early childhood. It is important that future research continues the investigation of cortisol metabolism and eating behaviours so that obesity-protective eating behaviours may be encouraged and unhealthy, stress-induced eating may be prevented.

Regression analyses in this chapter did not reveal any significant predictors for maternal reports of infant satiety responsiveness or food fussiness. However, greater enjoyment of food was predicted by an earlier age of introduction to solid food. This means that infants weaned later are perceived by their mothers to enjoy food less. This finding supports research by Möller, de Hoog, van Eijnsden, Gemke, and Vrijkotte (2013) who found that introducing infants to solid food after the age of 6-months was associated with mothers perceiving their infants to enjoy food less at 5-years-old.

In addition to this, Brown and Lee (2012) suggest that introducing infants to solid foods earlier may encourage them to consume larger amounts of energy, which may

in turn interfere with their self-regulation of intake. Research therefore suggests that earlier introduction of solid food may lead to increased energy intakes and overconsumption of food, through decreased self-regulation, and increased enjoyment, of eating. Future research should clarify this so that interventions can be designed that prevent mothers overriding their infants satiety signals, that help mothers to feed infants more sensitively and encourage infants to self-regulate their intake, during solid feeding. It should be considered here however, that in the current study, the average age infants were introduced to solid food was 20-weeks. It is therefore likely that results of this thesis demonstrate the effects of normal variation in weaning age.

However, it should also be acknowledged that increased enjoyment of food might just indicate a hungry baby who has been introduced to solid food early. For example, if a mother believes that milk alone is not fully satisfying her infant, she may introduce solid food early (McAndrew et al., 2010b). It is possible that 'hungry babies' elicit earlier weaning and will therefore already be predisposed to greater weight gain, even before they are weaned. In fact, previous research has demonstrated that earlier introduction to solids is associated with greater weight gain during infancy (Baird et al., 2008; Baker, Michaelsen, Rasmussen & Sorensen 2004; Forsyth, Ogston, Clark, Florey & Howie 1993; Kramer et al., 1985; Lande et al., 2005).

Regression analyses in the current study also revealed that slower infant weight gain was predicted by: an increased duration of breastfeeding, lower level of maternal education, maternal perception of lower infant enjoyment of food and food fussiness

and reduced clearance of cortisol via reduced 11β -HSD2 activity. Breastfeeding duration was the most significant predictor. These results suggest that infant eating behaviours and cortisol metabolism, alongside how long infants are breastfed for, are key predictors, over and above maternal feeding behaviours, of how much weight they will gain throughout the first year of life.

Previous research has found that breastfeeding is associated with slower weight gain between 3- to 6- and 6- to 9-months (Heinig et al., 1993) and positive eating behaviours (Brown & Lee, 2012; Kudlová & Schneidrová, 2012). Furthermore, results of Chapter 3 show that a longer duration of breastfeeding is associated with slower weight gain and maternal report of slower eating styles during the first year of life. Results of the current study support previous research by showing that slower infant weight gain from 1- to 12-months is predicted by an increased duration of breastfeeding.

The second strongest predictor of infant weight gain was maternal education, indicating that infants who gain weight more quickly have mothers who are more educated. This result was surprising because previous research has shown that infants from lower socioeconomic status (SES) families weigh more at 3-months and show greater weight gain from birth to 3-months, compared to those from higher SES families (Wijlaars, Johnson, van Jaarsveld & Wardle, 2011). This relationship is thought to arise because lower SES mothers are less likely to breastfeed, and breastfeed for shorter durations (Amir & Donath, 2008; Heck, Braveman, Cubbin,

Chavez & Kiely, 2006), and because breastfeeding is associated with slower weight gain in infancy (Heinig et al., 1993; Hörnell et al., 2013).

Results of the current study are thought to have arisen because of bias within the sample. Mothers who participated in the current study were significantly more educated (Office for National Statistics, 2011a) and breastfed for longer (52% versus 34% [McAndrew et al., 2010a]) than the national average. Future research should therefore seek to recruit a sample more representative of the UK population. A more socioeconomically diverse population is likely to support previous findings that lower SES infants will be heavier and gain more weight over the first year of life.

Slower weight gain was also predicted by maternal perceptions of lower food enjoyment and less fussiness. The finding that slower weight gain during infancy is predicted lower food enjoyment makes sense as it stands to reason that an infant who does not seem to enjoy food may be more difficult to feed and may therefore gain weight more slowly. This explanation is supported by research which has found that children who are perceived by their parents to enjoy food less and be less food responsive have lower BMIs at 4-years-old (Jansen et al., 2012).

The prediction of slower weight gain by maternal perception of lower food fussiness however, was unexpected. This is because previous research has found a negative relationship between parental perception of fussiness and BMI of their children (Jansen et al., 2012). In addition to this, infants with early established food refusal have been found to weigh less, during infancy and at 2-years-old, than infants who

do not have feeding problems (Lindberg, Bohlin, Hagekull & Thunström, 1994). It is therefore surprising that results of the current study show that greater weight gain is predicted by increased fussiness. One explanation for this finding is that mothers may feed infants they perceive as fussy, foods they know their infant likes. It is possible that when these 'fussy infants' are faced with a preferred food, they will consume more of it, which may in turn influence their weight gain.

As discussed in Chapter 4, research has shown that being exposed to glucocorticoids in utero is associated with low birth weight in human and animal models (Braun et al., 2013; Cleasby et al., 2000) and lower placental 11 β -HSD2 is associated with lower birth weight in human infants (Murphy et al., 2002; Stewart et al., 1995). Additionally, results of Chapter 4 are the first to suggest that reduced clearance of cortisol (via 11 β -HSD2) is associated with slower weight gain throughout the first year of life. Results of the current chapter support aforementioned findings and suggest that, even after accounting for other key predictors, increased exposure to cortisol, through lower clearance of it via 11 β -HSD2, is an independent and significant predictor of slower infant weight gain.

Results of this chapter appear contradictory in that reduced 11 β -HSD2 activity is associated with more observed food acceptances, yet is a predictor of slower weight gain. It is important to consider here that the meals consumed by infants during the observation were not standardised by the researcher. Therefore infants were offered different types, and different quantities, of food by mothers during the observed mealtime. In addition, plates were not weighed before and after the meal. Future

research should seek to clarify relationships between 11β -HSD2 activity and eating behaviours using a more standardised method of monitoring the amount of food accepted.

Despite such limitations, the current study is the first to provide evidence of relationships between cortisol metabolism and eating behaviours in infancy and has done so by using both maternal report of information and a naturalistic observation of a mealtime. Furthermore, it is the first study to show that even after accounting for key predictors such as breastfeeding duration, maternal BMI and mealtime behaviours and demographic factors, infant cortisol metabolism is an independent and significant predictor of both eating behaviours and weight gain during infancy.

Slower weight gain throughout the first year of life is predicted by a longer duration of breastfeeding, less food enjoyment and fussiness and reduced cortisol clearance (via 11β -HSD2). Future research should investigate whether these relationships persist into early childhood, post breastfeeding. Further research should also continue investigation of the possible relationships between cortisol metabolism and, both, healthful and obesogenic eating behaviours.

CHAPTER 7

GENERAL DISCUSSION

7.1 Aims and hypotheses of the thesis

The overall aim of the thesis was to investigate the roles of breastfeeding, positive maternal mealtime interactions and cortisol metabolism on weight gain and the development of eating behaviours during the first year of life. In order to achieve this, the first aim of the thesis was to replicate findings of previous research, which have found that longer durations of breastfeeding are associated with slower infant weight gain and healthier eating behaviours; this was achieved whilst accounting for key confounding variables and overcoming the issues of retrospective self-report of breastfeeding duration encountered by numerous studies.

The second set of aims focussed on the investigation of cortisol metabolism. Before this thesis, there was no published normative data on cortisol metabolism in infancy. Therefore the aims were to explore the development of infant cortisol metabolism and investigate the possible associations between maternal and infant cortisol metabolism and cortisol metabolism and bodyweight during the first year of life. It was hypothesised that there would be a positive relationship between maternal and infant cortisol metabolism and that there would be an association between infant cortisol metabolism and weight gain over the first year of life. Results of this thesis have supported these hypotheses by demonstrating associations between infant and

maternal 5 α -reductase activity and by discovering a relationship between infant weight gain and 11 β -HSD2 throughout the first year of life.

The thesis also aimed to investigate associations between breastfeeding duration, positive maternal mealtime interactions, cortisol metabolism and eating behaviours. Finally, the thesis then explored the key variables that predict infant eating behaviours at 12-months and weight gain over the first year of life. It was hypothesised that infants breastfed for longer durations would have mothers who display more positive interactions during mealtimes. These infants would have different patterns of cortisol metabolism and demonstrate eating behaviours that are obesity-protective. It was hypothesised that breastfeeding duration, positive maternal mealtime behaviours and steroid ratios indicative of cortisol metabolism would all be significant predictors of infant weight gain and eating behaviours at 12-months. These hypotheses were supported and results have shown that infants breastfed for longer durations eat more slowly, metabolise more cortisol and have mothers who interact more positively with them during an observed meal. Chapter 1, Figure 1.5 (page 56) illustrates relationships found in published research and those hypothesised in this thesis.

7.2 Summary of results

Figure 7.1 is a simplified diagram of the significant associations found in Chapters 3, 4, 5 and 6; a more complex version of this (Figure 7.2, page 219) can be seen after the summary of results section. Following this will be a critique of the methodology utilised throughout the thesis and implications for future research will be discussed.

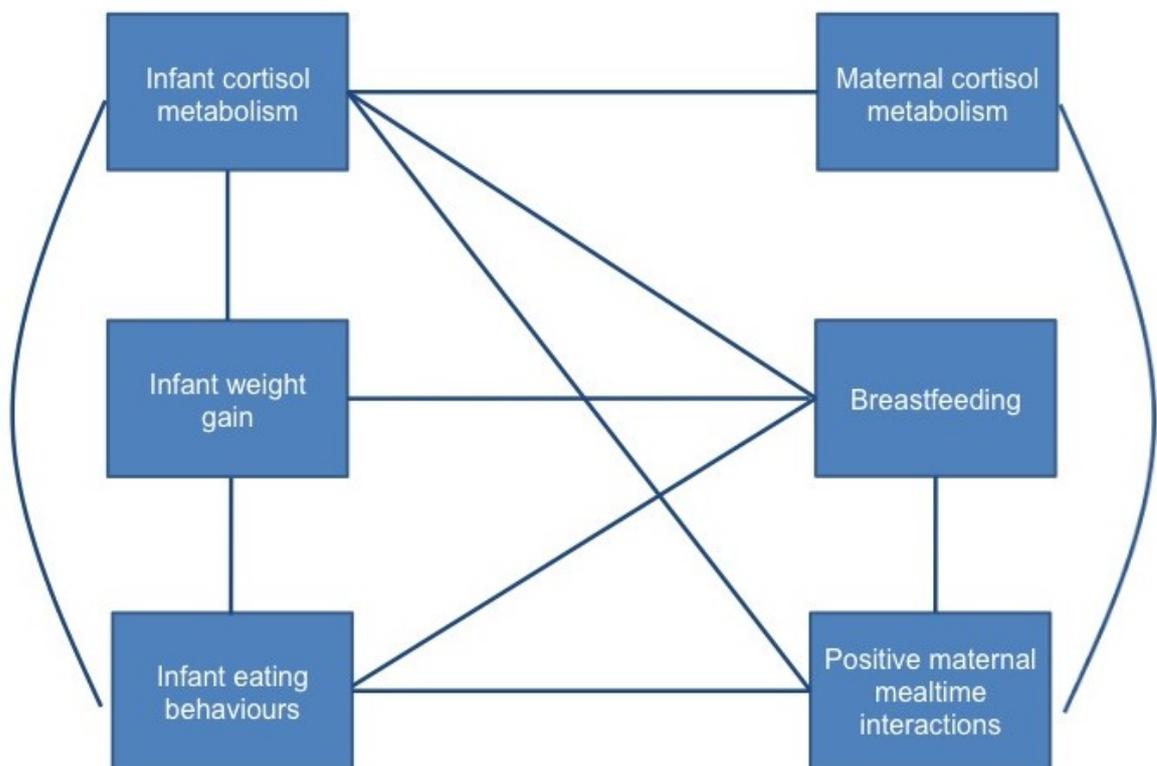


Figure 7.1

Simplified diagram of significant relationships between infant eating behaviours, weight gain, breastfeeding, cortisol metabolism and positive maternal mealtime behaviours

7.2.1 Breastfeeding

Chapter 3 demonstrated that a longer duration of breastfeeding was associated with lower infant weight SDS at 6- and 12-months and slower infant weight gain SDS from 1- to 6- and 1- to 12-months. Results suggest that breastfeeding has a dose-dependent effect on infant weight and weight gain during the first year of life, which appears to manifest particularly in the latter half of the first year. These results support previous research by Arenz et al., (2004), Harder et al., (2005), Hörnell et al.,

(2013), Kramer (1981), McCrory and Layte (2012) and Owen et al., (2005) who also found a dose-dependent effect of breastfeeding on later weight. The thesis was therefore successful in replicating the relationship between breastfeeding and weight gain.

Chapter 3 also demonstrated that infants who were breastfed for longer durations were perceived by their mothers to eat more slowly at 12-months-of-age. Chapter 3 proposed that breastfeeding may help infants develop a slower rate of eating as previous work has argued that breastfed infants work harder for their food (Cao et al., 2009) and better learn hunger and satiety cues (Birch & Fisher, 1998) than those fed from a bottle. This slower rate of eating, as perceived by mothers, may then contribute to slower weight gain and lower weight at 12-months. It was acknowledged however, that infants who gain less weight might do so due to having a smaller appetite, rather than because they were breastfed. Furthermore, it may be the case that mothers of infants who are slow eaters may breastfeed for longer due to having a less demanding or hungry baby.

Results of Chapter 3 also found that infants who were heavier at 12-months were perceived by their mothers to be less satiety responsive, to enjoy food more and to eat more quickly than lighter infants. The elimination of the relationship between weight and slowness in eating when controlling for breastfeeding duration suggests that breastfeeding may be a mechanism by which lighter infants develop slower eating styles. As explained above, this is likely to occur due to breastfed infants working harder for their food (Cao et al., 2009). In addition, breastfed infants

demonstrate less lower protein intakes than formula-fed infants (Whitehead, 1995) and have also been found to consume less milk if it contains more fat (Tyson et al., 1992). Considering the points made above, however, infants breastfed for longer durations may be less hungry or demanding and may have smaller appetites than those breastfed for shorter durations or not at all. Therefore the disappearance of the relationship between weight and slowness in eating may be due to inadvertently controlling for smaller appetite rather than the relationship being a direct effect of breastfeeding duration.

Results of Chapter 5 show at 6-months postpartum, a longer duration of breastfeeding was associated with greater maternal appropriateness and sensitivity during an observed mealtime; at 12-months, a longer duration of breastfeeding was associated with increased positive maternal vocalisations, appropriateness and sensitivity during an observed mealtime. It is suggested that as these relationships become stronger over time, it is possible that breastfeeding increases sensitive maternal behaviours. However, it is also true that more sensitive mothers choose to breastfeed; breastfeeding beyond 6-months may therefore be a practice that only the most sensitive mothers do. Furthermore, this thesis cannot address whether or not sensitive maternal behaviours are enhanced through breastfeeding, as there was no baseline information regarding maternal sensitivity or other positive interactions.

7.2.2 Cortisol metabolism

Results of Chapter 4 revealed that maternal steroid ratios indicative of 5α -reductase activity show significant changes over the course of the first postnatal year. Activity appeared very low at 1-week postpartum, increased significantly between 1-week and 1-month and 1- and 3-months, slowed between 3- and 6-months and then stabilised from 6- to 12-months. It is not yet known how changes in 5α -reductase occur, or what these changes may mean; although it is known that 5α -reductase activity is associated with weight gain, weight loss, markers of insulin function and sensitivity and it affects cortisol availability and androgen generation (Andrew et al., 1998; Nelson et al., 1999; Schmidt et al., 2006; Tomlinson et al., 2008; Vassiliadi et al., 2009). However, it is not known if these effects are causes or consequences of changes in 5α -reductase activity. This thesis has provided the first normative set of data describing maternal cortisol metabolism, in a non-clinical sample, throughout the immediate postnatal period and first postpartum year.

Analysis of infant steroid ratios demonstrated that there are significant differences in infant cortisol metabolism over time and between male and female infants across the first year of life. Ratios indicative of cortisol activation (via 11β -HSD1) increased significantly between 3- and 6-months and stabilised around 12-months. Ratios indicative of cortisol clearance (via 5α -reductase) peaked at 3-months and decreased at 6- and 12-months. Boys appeared to be activating and clearing more cortisol than girls at 3-months, but not at any other time point. As previously mentioned, it is unknown how changes in 5α -reductase arise, and what such changes may mean.

However, 5 α -reductase does appear to be involved in androgen generation, through activation of dihydrotestosterone; this role is likely to be equally as important as its effect on cortisol availability.

Conversely, infant steroid ratios indicative of 11 β -HSD2 suggest that cortisol clearance via this enzyme decreased over the first year of life and most significantly between 6- and 12-months. There were no significant gender differences in 11 β -HSD2 activity at any time point during the first year of life. Changes in steroid ratios of human adults result in changes in metabolic phenotype and alterations to weight and insulin sensitivity (Tomlinson et al., 2008). This thesis cannot draw any firm conclusions as to whether this is also the case during infancy, although it is possible. To fully examine metabolic phenotype during infancy, blood samples would have been required in order to obtain measurements of insulin and glucose. Despite this however, this thesis has provided the first set of normative data on cortisol metabolism in human infants. Furthermore, it is the first data set to show there are gender differences in ratios indicative of cortisol activation (via 11 β -HSD1) and clearance (via 5 α -reductase, [but not 11 β -HSD2]) in human infants during the first year of life.

This thesis has also provided the first evidence for a relationship between cortisol metabolism and weight gain in infancy. Results of Chapter 4 revealed that infants who clear less cortisol, via 11 β -HSD2, exhibit slower weight gain from 1- to 12-months. Previous research has found that exposure to greater levels of cortisol in utero, through reduced clearance rates of it via 11 β -HSD2 in the placenta, is

associated with lower birth weight in human infants (Murphy et al., 2002; Stewart et al., 1995). In addition to this, AME, a condition associated with low birth weight, is caused by deficits in 11 β -HSD2. Results of Chapter 4 lend support to aforementioned research and suggest that greater exposure to cortisol in the postnatal period (through reduced clearance of it) is associated with slower weight gain even within the limits of healthy weight gain in healthy infants.

Results of this thesis have not found relationships between weight gain and activity of 11 β -HSD1 or 5 α -reductase during the first year of life. However, this does not mean that early changes in these ratios will not have effects later on in childhood. For example, given that 5 α -reductase activity is associated with insulin sensitivity and action, it is possible that changes in 5 α -reductase activity in infancy may have effects on later weight, and alterations in insulin action or sensitivity could be a potential mechanism by which this occurs.

Results of Chapter 6 have provided the first evidence for a relationship between cortisol metabolism and eating behaviours in human infants. Findings have shown that infants who clear more cortisol via 5 α -reductase are perceived by their mothers to eat more slowly at 12-months. Interestingly, breastfeeding duration did not have an effect on this relationship. This suggests that regardless of whether or not mothers choose to breastfeed, and how long they breastfeed for, infants who clear more cortisol (via 5 α -reductase) eat more slowly than those who have steroid ratios indicative of less cortisol clearance. This thesis has therefore provided the first

evidence that increased 5 α -reductase activity is associated with slower eating styles, which are likely to be protective of obesity later in life.

Results of Chapter 6 have also shown that infants who clear less cortisol, via 11 β -HSD2, are observed to accept more food during a meal. This suggests that increased exposure to cortisol, through a limited clearance of it, is associated with eating more during a meal. Although it initially may seem contradictory to the finding that reduced cortisol clearance, via 11 β -HSD2, is associated with slower weight gain, findings of this thesis do fit with published research. Such research has found increased exposure to cortisol limits early pre- and postnatal growth (Mune et al., 1995; Reynolds, 2010), yet promotes increased food and caloric intake in human adults (Epel et al., 2001; Tataranni et al., 1996; Wolkowitz et al., 2001). As discussed in Chapter 6 (section 6.4), it may also be the case that infants who eat more during a meal may snack less between meals. Snacking less between meals may thus prevent overconsumption of high calorie food and excessive weight gain. Follow up of this cohort of infants into later childhood may help to explore this potential mechanism.

Results of Chapter 4 also found positive associations between infant and maternal ratios also indicative of cortisol clearance, via 5 α -reductase, throughout the first postnatal year. This thesis has provided the first evidence of relationships between infant and maternal cortisol metabolism in a sample of healthy mothers and their healthy infants. Worthy of note is that these associations remain true after controlling for breastfeeding – a feeding method likely to expose the infant to maternal levels of

cortisol. Whilst the enzymes responsible for cortisol metabolism would not be transmitted through breast milk, it is speculated that other maternal factors may programme their infant's levels of metabolising enzymes through genetics and/or sensitive behaviour.

Chapter 5 demonstrated that although breastfeeding duration was not related to maternal ratios indicative of cortisol metabolism, it was positively associated with infant 5α -reductase activity at 6- and 12-months and 11β -HSD1 activity at 12-months. Findings have shown that whether mothers breastfeed their infants, or not, has no association with their own cortisol metabolism. Results of this thesis have also demonstrated that breastfeeding duration is associated with increased cortisol activation in infants at 12-months and increased cortisol clearance in infants at 6- and 12-months. It is therefore possible that the increased metabolism of cortisol seen in infants breastfed for longer durations may help programme their HPA axis and regulate their stress response. This thesis has provided the first evidence of the relationships between breastfeeding and infant cortisol metabolism, thus strengthening the weight of the argument that maternal behaviour coordinates infant psychobiological function during the first year of life (Spangler et al., 1994).

7.2.3 Positive maternal mealtime interactions

Results of Chapter 5 reveal that more sensitive mothers clear more cortisol via 11β -HSD2 at 12-months. This is in line with previous research, which has shown that depression is associated with withdrawn (not sensitive) parenting behaviour (Murray

et al., 2010) and increased levels of cortisol (Nemeroff & Vale, 2005). Increased clearance of cortisol is therefore seen as positive and adaptive for human health and behaviour.

Results of Chapter 5 also demonstrate that mothers who vocalise positively to their infant during mealtimes have infants who activate (via 11β -HSD1) and clear (via 5α -reductase) more cortisol at 12-months; mothers who are deemed more appropriate during meals have infants who activate more cortisol at 12-months. However, when breastfeeding was controlled, relationships between cortisol activation and maternal interactions were no longer significant. Results of this thesis therefore suggest that breastfeeding duration appears to explain at least some of the relationship between infant cortisol activation and positive maternal mealtime interactions. Although causality cannot be inferred from these analyses, it is possible that the increased activation of cortisol, seen in infants who are breastfed and whose mothers interact positively with them, may have an adaptive role in the programming of their HPA-axis. Furthermore, this thesis has demonstrated that significant components of an infant's social/emotional environment are involved in their cortisol metabolism, which again strengthens the argument that maternal behaviour has an organisational effect on their offspring's psychobiological function during infancy (Spangler et al., 1994).

Results of Chapter 6 show that greater positive maternal vocalisations, appropriateness and sensitivity during a meal are associated with fewer observed food acceptances during a meal. As discussed in Chapter 6 (section 6.4), Worobey et al., (2009) found that mothers who regularly overfed their infants were less

sensitive to their infant's cues. Results of this thesis lend support to Worobey et al., (2009) and suggest that mothers who display more positive interactions during feeding may be less likely to overfeed their infants. In addition, results of this thesis have shown that mothers who are observed to be less appropriate during a meal report having infants who are fussy with food. It is interesting that mothers observed to be less appropriate have infants who they perceive as fussy, yet accept more food during a meal; it is suggested that this may be because if a mother feeds a preferred food to a fussy child, they may eat lots of it. The association found between less appropriate maternal behaviour and increased infant fussiness support previous research by Farrow (2005), who found that mothers who display more inappropriate behaviour during a meal report experiencing more mealtime negativity. Although causality cannot be inferred from correlational analyses, Powell et al., (2011) propose that there is a bi-directional relationship between insensitive maternal feeding practices and an infant's food avoidance behaviours; parents of food avoidant children may pressure them to eat more during a meal, and pressuring feeding behaviours may cause or exacerbate the development of feeding problems in the child.

7.2.4 Predictors of eating behaviours and weight gain

Regression analyses were used to establish the best predictors, out of those explored throughout the thesis, of weight gain and eating behaviours at 12-months. Regression analyses in Chapter 6 revealed that: greater enjoyment of food was predicted by an earlier age of introduction to solid food; slowness in eating was

predicted by increased cortisol clearance via 5α -reductase; and more food acceptances during a meal were predicted by less cortisol clearance, via 11β -HSD2.

The prediction of greater food enjoyment by earlier age of introduction to solid food supports research by Möller et al., (2013), who found that introducing infants to solid food after the age of 6-months was associated with mothers perceiving their infants to enjoy food less at 5-years-old. Combined with findings of Brown and Lee (2012) it is possible that earlier introduction to solid food may lead infants to consume energy intakes above those required, through decreased self-regulation and increased enjoyment of eating. This may then predispose infants to excess weight gain.

It is also important to consider here however, that increased enjoyment of food might just indicate a hungry baby who was introduced to solid food earlier. If a mother thinks she has a hungry baby, she may believe that milk is not fully satisfying her infant and may then introduce solid food early. It is likely that these 'hungry babies' often elicit earlier weaning and will therefore already be predisposed to greater weight gain, even before they are weaned. Previous research has demonstrated that one of the key reasons for introducing infants to solid food is the perception that they are no longer satisfied with milk alone (McAndrew et al., 2010b) and that earlier introduction to solids is associated with greater weight gain during infancy (Baird et al., 2008; Baker et al., 2004; Forsyth et al., 1993; Kramer et al., 1985; Lande et al., 2005).

The prediction of slowness in eating by increased 5α -reductase activity, and food acceptances by reduced 11β -HSD2 activity, implies that increased exposure to cortisol, through a limited clearance of it, predicts faster eating styles and more food acceptances within a meal. Such findings therefore highlight that cortisol metabolism is a primary predictor of specific infant eating behaviours at 12-months, over and above other key variables of interest, including: weight gain, breastfeeding, positive maternal mealtime behaviours and demographics. Such findings appear supportive of Epel et al., (2001) who found that adult women who showed greater cortisol increases (in response to stress) consumed more calories than those who showed smaller cortisol increases.

It must also be considered however, that prolonged exposure to cortisol, in response to stress, may eventually reduce sensitivity to satiety signals and affect the reward system; this may lead to increased consumption of highly palatable food, contributing to fat distribution and weight gain (Adam & Epel, 2007). Although the current study did not look at cortisol in response to stress, the long-term implications for eating behaviours of increased exposure to cortisol during infancy require further investigation.

Regression analyses in Chapter 6 also found that slower infant weight gain is predicted by: an increased duration of breastfeeding, lower level of maternal education, reduced food enjoyment and fussiness (as perceived by the mother) and reduced clearance of cortisol via reduced 11β -HSD2 activity. Results therefore confirmed hypotheses that longer durations of breastfeeding would predict slower

weight gain, thereby supporting findings of Arenz et al., (2004), Harder et al., (2005), Hörnell et al., (2013), Kramer (1981), McCrory and Layte (2012) and Owen et al., (2005).

The prediction of slower infant weight gain by lower maternal education, however, was unexpected. Previous research has shown that infants from lower SES families gain more weight compared to those from higher SES families (Wijlaars et al., 2011). As described in Chapter 6 (section 6.4), participating mothers were significantly more educated (Office for National Statistics, 2011a) and breastfed their infants for longer (McAndrew et al., 2010a) than the national average. The finding that lower maternal education predicts slower infant weight gain is therefore believed to be spurious and to have arisen because of bias within the sample.

Although positive maternal mealtime interactions are significantly associated with breastfeeding duration and infant cortisol metabolism and eating behaviours, regression analyses have shown that they do not independently predict eating behaviours at 12-months or weight gain during infancy. Results of regression analyses therefore suggest that breastfeeding duration, infant eating behaviours and cortisol metabolism are the key predictors, over and above positive maternal feeding behaviours, of how much weight infants will gain throughout the first year of life.

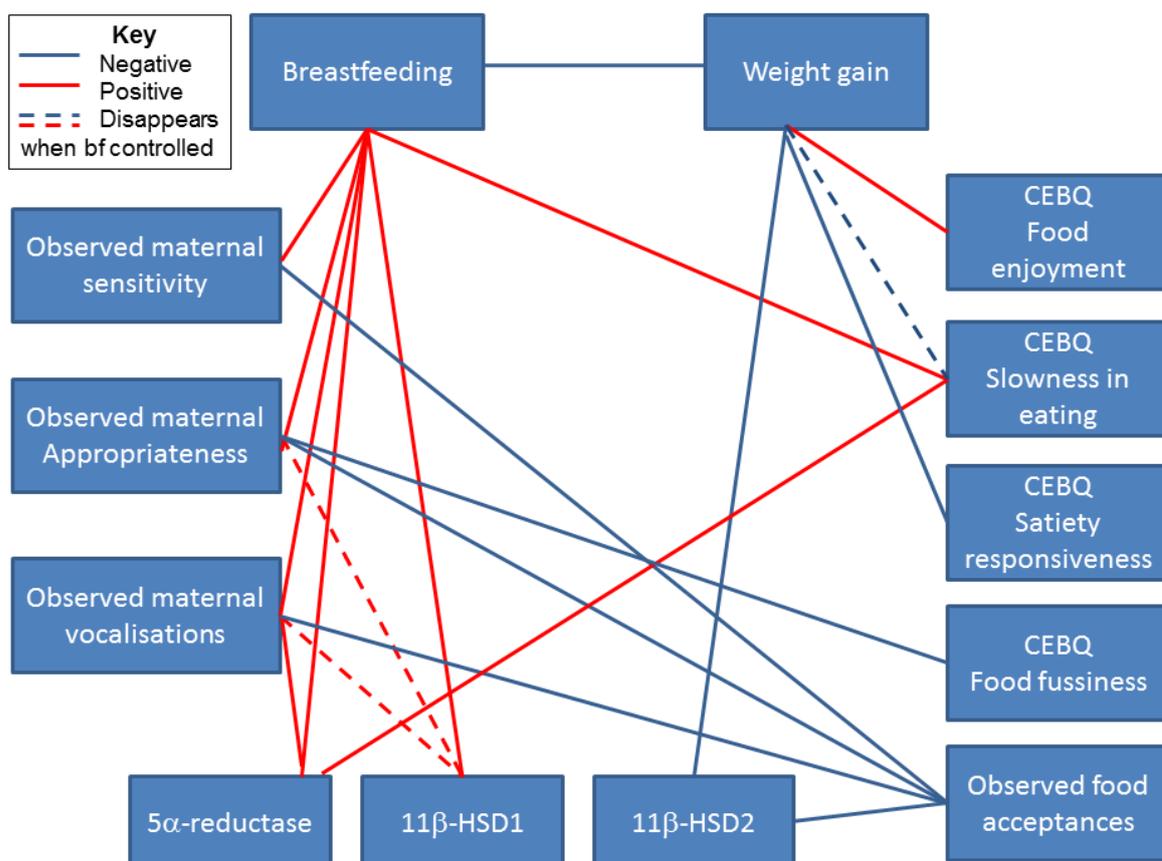


Figure 7.2

Significant relationships between infant eating behaviours (as reported with the CEBQ), observed food acceptances, weight gain, breastfeeding, cortisol metabolism and positive maternal mealtime behaviours

7.3 Methodological critique and future directions

One of the primary strengths of this thesis is its longitudinal design. Mother-infant dyads were visited at home in the immediate postnatal period and several more times over the first year; this enabled the quick establishment of a good rapport between the researcher and participants. The longitudinal design also ensured accurate recording of breastfeeding duration, measurement of weight and allowed

the novel investigation of the development of cortisol metabolism throughout the first year of life.

Problems encountered by previous studies, such as the lack of control of covariates and the retrospective self-report of information, were mainly overcome in this thesis. Analyses throughout the thesis controlled for a range of covariates, including: maternal age, education, BMI, number of cigarettes smoked during pregnancy, household income, infant birth weight SDS and age introduced to solids. It would be of interest for future research in this area to separate infants exclusively breastfed for at least 4-months from those fed a mixture of breast and formula milk. If breastfeeding exclusivity could be assessed in addition to duration, stronger relationships with eating behaviours and weight gain may be seen. The sample recruited for this thesis was unfortunately not large enough to do this at each time point during the study.

Continued research in this area is needed in order to enhance understanding of breastfeeding's effect on the development of obesity-protective eating behaviours. It is possible that breastfeeding influences the development of slower eating styles, which may in turn enhance satiety responsiveness in early childhood. This is important, as previous research has found that greater satiety responsiveness is related to a lower risk of being overweight in childhood (Webber et al., 2009). Research such as this will inevitably further our understanding of breastfeeding's protective role against overweight and obesity in childhood. A final follow-up visit when the children are aged between 2- and 3-years would begin to address

questions regarding whether the associations found in this thesis persist into early childhood; these visits are currently being undertaken.

This thesis is of course, not without its limitations. Firstly, as discussed in Chapter 3 (section 3.4), although participants were from a variety of demographic, socioeconomic and cultural backgrounds, the educational level achieved by mothers in the current study was significantly higher than the national average (Office for National Statistics, 2011a). Sixty-three percent of the participants recruited either had a degree or another professional qualification. Furthermore, a higher proportion of women in the current study were breastfeeding at 6-months compared to the UK average (52% versus 34% [McAndrew et al., 2010a]). Although these factors were controlled for in analyses, future research should seek to replicate findings of this thesis in a more socioeconomically diverse sample that is more representative of the UK population.

It is important to note here however, that the sample recruited for this thesis was representative of the age of mothers giving birth in England and Wales. Compared to 2010 data stating that infants were most likely to be born to mothers between the ages of 25- and 34-years (Office for National Statistics, 2011b), the mean age of mothers recruited into this study was 29.4 years.

Another limitation of this research concerns the self-selected nature of the sample. Although women from a wide range of socioeconomic and cultural backgrounds were approached and informed about the study, a bias may have occurred in mothers who

agreed to participate. This means that mothers with a particular interest in breastfeeding and infant weight gain may have been more likely to participate. It is possible that compensating mothers for their time, with high street or book vouchers for example, may have aided recruitment and resulted in a more diverse sample.

Another strength of this thesis is that maternal behaviours were assessed through naturalistic observations of mealtimes at 6- and 12-months postpartum. Mothers were observed feeding their infants in their own homes whilst the researcher sat in another room, wherever possible. This method is preferable to maternal self-report of behaviours, and is likely to have resulted in the observation of more representative mealtimes and feeding interactions than if participants would have come into the lab. In addition to this, participants had already met and conversed with the researcher numerous times before they were observed feeding their infant. This is likely to have made participants feel more at ease and comfortable when being recorded.

It is important to take into account here however, that the meals consumed by infants during the observation were not standardised by the researcher. This meant that mothers presented their infants with a range of different food types and portion sizes during the observed mealtimes. In addition, plates were not weighed before and after the meal. Although these methods may have given a more accurate measure of the quantity of food consumed, they inevitably would have reduced the representativeness of the mealtime, and perhaps even, the ease at which participants were recorded. In spite of this, future research in this area should seek to use a more standardised method of monitoring the amount of food accepted.

A further strength of this study was that the procedure of collecting urine and nappy samples for the analysis of cortisol metabolism was not an invasive one. Obtaining blood samples is an invasive procedure that is not without complications. In addition, it is a stressful procedure and undoubtedly influences measured cortisol levels. Isolated circulating cortisol levels are of limited value in assessing the HPA axis, they are highly variable, influenced by many factors including stress and are critically dependent upon the time of day of sampling. As such 24-hour urine measurements provide a global measure of both adrenal glucocorticoid output as well as the pathways of glucocorticoid metabolism.

In addition to plasma, breast milk samples were also not collected. Samples of breast milk would have enabled the investigation of its steroid and nutrient content and how it varies throughout any given day, at different postnatal stages and between mothers. However, for this thesis, only resources for urinary analysis were available.

Finally, the current study recruited a sample of women who had no complications, such as gestational diabetes, during pregnancy and infants were born full-term of a healthy weight. No medical conditions were reported for mother or infant and it can therefore be said with confidence that the current study has achieved its aim in providing the first set of normative data on the metabolism of cortisol in healthy human infants. However, this does limit interpretation of these findings to this group and cannot be extrapolated to overweight, underweight, or growth impaired infants, groups in whom exploration of cortisol metabolism, weight gain and eating behaviours may be particularly fruitful.

Future research should seek to replicate the associations found between cortisol metabolism and weight gain, in premature and infants born small for gestational age (SGA). This group of infants can show rapid catch-up growth in early infancy – a pattern of weight gain that has been found to predict obesity later in life (Cole, 2007; Lanigan & Singhal, 2009; Taveras et al., 2009). Investigating cortisol metabolism and weight gain in premature and SGA infants is therefore likely to enhance understanding of the contributors to excessive weight in infancy. It may also help increase the knowledge base required to develop effective preventions and interventions of paediatric obesity.

As highlighted in Chapters 4 and 5, it cannot be guaranteed that all nappy samples were 24-hour collections. This is because mothers were requested to dispose of any soiled nappies. Mothers may have also supplied nappies, unknowingly, that were unusable. For example, when slightly soiled or when they did not contain enough urine to be extracted. In addition, mothers may have also forgotten to start collecting at the correct time of day. It is therefore inevitable that some nappies will have been 'missed'. In the attempt to prevent this being too much of an issue, however, participants were reminded to start collecting nappies the day before the visit took place by the researcher. Furthermore, when absolute steroid values were plotted by the laboratory technician, the values resembled what would be expected from a 24-hour sample.

A further limitation of the thesis involves the use of parametric statistics with data that is not normally distributed. The following variables did not follow a normal distribution:

breastfeeding duration, demographic factors, maternal postnatal depression and most steroid ratios. Using parametric tests on this data may therefore cause inaccuracies in results. However, where necessary, parametric tests were used because infant weight, weight gain and eating behaviours were normally distributed and it was an essential aspect of the study to investigate potential relationships between variables whilst controlling for covariates. In addition to this, based on the number of predictors used and the sample size of the study, some of the regressions in Chapter 6 were low in power. This means that analyses used in this thesis may not have detected subtle effects of variables on this population of mothers and their newborn infants.

7.4 Implications

Breastfeeding is significantly associated with slower eating styles and slower weight gain during the first year of life. Breastfeeding for longer durations is therefore likely to aid the development of obesity-protective eating behaviours through enabling infants to learn to attend to their internal hunger and satiety signals. A longer duration of breastfeeding is also associated with increased cortisol metabolism at 6- and 12-months. As prolonged exposure to circulating cortisol can have negative effects on HPA-axis functioning and promote overeating in humans, an increased metabolism of cortisol is considered to be healthy and adaptive. Longer durations of breastfeeding are therefore likely to help: (1) regulate weight gain and eating behaviours; and (2) programme a healthy stress response and HPA axis functioning, during infancy. As rates of breastfeeding in the UK are currently lower than desired, it

is important to continue the promotion of breastfeeding, and improve support available for breastfeeding mothers. It is predicted that strategies such as these are likely to result in significant benefits for health, including the development of obesity-protective eating behaviours, slower weight gain and healthy function of the HPA axis.

Increased positive maternal vocalisations, appropriateness and sensitivity are associated with fewer food acceptances during an observed mealtime and longer durations of breastfeeding. Furthermore, irrespective of whether or not mothers choose to breastfeed, or how long they breastfeed for, those observed to be more appropriate during feeding are more likely to have an infant they perceive not to be fussy with food. Mothers who interact with their infants more positively during meals are therefore less likely to encounter feeding problems and are less likely to overfeed their infants. Results of this thesis highlight the importance of developing interventions that will help and encourage mothers to interact more positively and sensitively with their infants during feeding. Furthermore, findings emphasise that irrespective of whether or not mothers choose to breastfeed, mealtime interactions are still important and it is essential that mothers receive support with feeding their infants sensitively. Behaving more sensitively will help mothers more accurately interpret and respond to their infant's communications and prevent them from overriding their infant's internal hunger and satiety cues. Infants will therefore better learn these internal cues themselves, which will help them to develop slower eating styles that are more satiety responsive and prevent excess weight gain.

Results of this thesis have shown that more sensitive mothers clear more cortisol, via 11 β -HSD2. Furthermore, mothers who vocalise more positively during feeding have infants who clear more cortisol at 12-months. This relationship remains true even after controlling for breastfeeding, which suggests that there are significant components of an infant's environment involved in their cortisol metabolism. As explained above, increased clearance of cortisol is considered to be healthy and adaptive and may help to regulate HPA axis function during infancy. It is therefore speculated that interventions that aim to help mothers interact more positively with their infants, may have significant benefits for HPA axis function. Sensitive maternal behaviour may programme levels of metabolising enzymes in infants, which may then influence healthy regulation of the HPA axis.

Results of analyses investigating cortisol metabolism and weight gain suggest that increased glucocorticoid exposure, through limited clearance of it (via 11 β -HSD2), may limit growth. Conversely, results failed to find relationships between weight gain and 11 β -HSD1 or 5 α -reductase activity. From these findings, it cannot be concluded whether early postnatal changes in steroid ratios have effects later on in childhood. However, it is possible that alterations in steroid ratios during infancy could affect later weight gain through their association with insulin sensitivity. Further longitudinal studies are essential in order to: (1) establish whether changes in steroid ratios during infancy have effects in later childhood; (2) explore whether these effects could be mediated by increased positive parental interactions; and (3) investigate whether the inverse relationship between 11 β -HSD2 and weight is maintained into early

childhood and to understand the relevance of this relationship to infants born premature or small for gestational age.

7.5 Conclusion

Despite inevitable limitations, this thesis has made a valuable contribution to the literature investigating weight gain and eating behaviours in infancy. The thesis has provided the first set of normative data on the development of cortisol metabolism throughout the first year of life and has highlighted the existence of a relationship between cortisol metabolism and weight gain in infancy. By demonstrating a relationship between positive maternal interactions and infant steroid ratios, it has proposed that significant aspects of an infant's environment are involved in the metabolism of cortisol. Furthermore, this thesis has provided the first evidence that infant cortisol metabolism is an independent and significant predictor of specific infant eating behaviours and weight gain in the first year of life, even after accounting for key predictors such as breastfeeding duration and maternal interactions during feeding. Future research should investigate the long-term effects of reduced cortisol clearance on weight and weight gain in infancy and early childhood. It is important that future research also investigates the potential mechanisms underlying the relationship between cortisol metabolism, weight and eating; currently, such mechanisms are not yet known.

The thesis has successfully replicated the relationship between breastfeeding and weight gain, whilst using a sound methodology and accounting for a range of

covariates, and has suggested that breastfeeding duration may explain at least part of the relationship between infant cortisol metabolism and positive maternal interactions. Future research should continue incorporating the investigation of bioactive factors in breast milk and maternal behaviours during feeding to enhance understanding of how breastfeeding influences the development of eating behaviours during infancy. Current breastfeeding rates are lower than that desired in the UK; strategies to promote breastfeeding could therefore result in significant benefits for health.

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APPENDICES

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APPENDIX 1

UK-WHO Growth Charts









APPENDIX 2

Participant information sheet



UNIVERSITY OF
BIRMINGHAM

Participant Information Sheet

Title of study: Breastfeeding, cortisol, infant growth and eating.

We would like to invite you to take part in our research study. Before you decide, we would like you to understand why the research is being done and what it would involve for you. One of our team will go through the information sheet with you and answer any questions you have. This should take about 15 minutes. Your participation in this study is voluntary. If you do agree to take part, we will then ask you to sign a consent form. You are free to withdraw at any time, without giving a reason.

What is the study about?

As part of the Infant and Child Laboratory in the School of Psychology, University of Birmingham, we are interested in children's eating behaviours and weight gain during their first year of life. We are also interested in how their eating behaviours are related to other factors such as breastfeeding, formula-feeding and how they interact with you during mealtimes. We are also starting to look at more biological factors (such as cortisol, a hormone involved in blood sugar regulation) and their impact on eating behaviours. We cannot obtain all the information we need through questionnaires alone, so we also visit you at home and record how your child interacts with you during a mealtime. In addition to this, we ask you for 24 hours worth of used nappies to test for your infant's level of cortisol.

What will my child and I be asked to do?

When the researcher speaks to you on the maternity ward, she will give you an information sheet and explain about the study and ask if you have any questions. She will then ask your permission to call you within the next couple of days to ask you whether you would definitely like to participate. If you would still like to take part in this study then she will first visit you at home when your infant is one week old. During this visit she will collect a 24-hour sample of used nappies (to determine infant cortisol), and a urine sample from yourself to measure your cortisol. If it is possible, a 24-hour urine sample from yourself would be extremely beneficial to the study. However, if it is not possible then a single urine sample would be greatly appreciated. During the 1 week visit, the researcher will also measure your infant's weight and length, and your weight and height and you will be requested to complete a questionnaire about some background information and feeding information. Before each visit, she will also ask you to complete a feeding diary. This diary will need completing every day (for seven days) and it will ask whether you are breast or

formula feeding your baby and how long your baby feeds for. Although this diary requires regular entries, it is very quick and easy to complete.

When your infant is 1 month old, the researcher will visit you at home for a second time and will measure your infant's weight and length and your weight. She will also collect a 24-hour sample of your baby's used nappies and a small urine sample from yourself. You will also be requested to complete questionnaires regarding feeding and how you are feeling since giving birth. When your infant is 3 months old, the researcher will contact you via telephone to discuss feeding. About eight days before she calls you, a questionnaire will be posted to you along with a freepost envelope for you to complete and return to us. She will also collect a 24-hour sample of used nappies (to determine infant cortisol level), and either a small urine sample, or a 24 hour collection of urine from yourself, whichever you prefer.

When your infant is 6 months old, the researcher will visit you at home for the third time. She will measure your infant's weight and length and your weight. She will also collect a 24-hour sample of used nappies and a urine sample from yourself. You will be requested to complete a couple of questionnaires about feeding and the behaviour of your infant. You will also be recorded whilst feeding your infant solid foods to see how your child interacts with you during a mealtime. You will only be asked to do this when you have already begun the weaning process. You will be asked to feed your infant as usual and recording will begin when you first offer food to your infant and will cease when food is removed.

The fourth and final visit will take place when your infant is aged 12 months. The researcher will come to your home and measure your infant's weight and length and your weight. She will also collect a 24-hour sample of used nappies and a urine sample from yourself. You will be requested to complete questionnaires regarding feeding, your infant's eating behaviours, character and about your own eating behaviours and feelings. You will also be recorded again whilst feeding your infant solid food to see how your child interacts with you during a mealtime.

The table below briefly describes what each visit will involve and approximately how long each visit will last.

Time Point	What will the researcher collect?	Estimated length of visit
1-week home visit	24-hour sample of used nappies	45 minutes
	Maternal urine sample	
	Infant weight and length and maternal weight and height	
	Two short questionnaires asking about feeding and some background information	
1-month home visit	24-hour sample of used nappies	35 minutes
	Maternal urine sample	
	Infant weight and length and maternal weight	
	Two short questionnaires asking about feeding and how you are feeling since giving birth	
3-month phone call	24-hour sample of used nappies	20 minutes
	Maternal urine sample	
	Three questionnaires asking about feeding and current health	
6-month home visit	24-hour sample of used nappies	1 hour 30 minutes
	Maternal urine sample	
	Infant weight and length and maternal weight	
	Five questionnaires asking about your infant's characteristics, feeding, current health and how you are feeling since giving birth.	
	Mealtime observation	
12-month home visit	24-hour sample of used nappies	2 hours
	Maternal urine sample	
	Infant weight and length and maternal weight	
	Nine questionnaires asking about your infant's characteristics, eating behaviours, feeding, current health and how you are feeling since giving birth.	
	Mealtime observation	

Who can take part?

Participation in this study is voluntary. You can take part in the study if you have safely given birth in hospital to a healthy full-term baby. Participants should live within easy travelling distance of the University of Birmingham (usually no more than 15 miles away). Finally, if you cannot read or write in English, unfortunately you will not be able to take part in this particular study.

What are the benefits of the research?

There are no major benefits to you for taking part in this study. However your participation may help us to further understand formula- and breastfeeding's effect on eating behaviours and weight gain in the first year of life. Your participation may also help us to discover ways to encourage the development of healthy eating behaviours from the first year of age onwards. You will not need to travel anywhere to take part in this study as all visits take place either when the researcher visits you at home or over the telephone.

What are the risks of taking part in this research?

There are very few risks associated with this research. All researchers within the Infant and Child Laboratory are CRB checked. Some of the questionnaires that we ask you to complete may raise concerns for you regarding eating behaviours and psychological wellbeing. Similarly, when we weigh and measure your child and yourself, you may have some concerns regarding feeding or weight. You can speak to a member of the research team if you are concerned about this. Sources of support for eating problems and parenting will be listed for you in a sheet to keep at home should you wish to refer back to it at a later time.

What happens to the information I provide to you during the study?

All the information you provide to us is private and confidential. We give number codes to all participants and never store names or contact details with the study data you provide. We do publish scientific articles using the data you provide but it is never possible to identify an individual.

The study cannot be anonymous because we store video-recordings of you and your child. However, these video-recordings are kept securely on a computer hard disc, which is kept in a locked room in the laboratory. A CD copy is kept in a locked cabinet in the researcher's office at the University of Birmingham. Only researchers directly involved in this study can access the recordings. Recordings are kept for 10 years, then the discs are destroyed. The recordings on the hard-drive of the computer will be wiped after 10 years.

Your questionnaire data is also kept in a locked cabinet in the laboratory. This raw data is kept for 10 years then shredded. Databases of the raw data are also made and are kept on computers. Only the person in charge of the study and the research team, have access to the databases. Databases will also be deleted after 10 years.

Urine samples will be stored in freezers in the institute of biomedical research, University of Birmingham for 10 years. Only those individuals directly involved with the research will have access to the samples. Samples will be stored with a numeric

code with which it will be possible to identify particular participants who may either need to be contacted or if they wish to withdraw their data from the research. It is possible that we may wish to use these samples again in future research. We will ask your permission to do so on the consent form.

Confidentiality may be breached if you reveal information that suggests you are at risk of harm to yourself or another person. If this occurs, appropriate sources of support will be notified. However, should this occur, we will always inform you first of our actions.

What if a problem is detected in the urine sample provided by myself or my infant?

If the urine sample raises a concern regarding the health of you or your baby, an outpatient appointment will be made for you with an Endocrinologist. If this was to occur, you would be contacted by telephone and letter and offered an appointment to come and discuss the results at a face-to-face appointment and appropriate clinical care will be given.

Will you inform my doctor that I am taking part?

We will not need to inform your GP, however with your permission, we will advise your health visitor that you and your baby are taking part in this study.

Who has reviewed the study?

All research in the NHS is looked at by independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given favourable opinion by Birmingham, East, North and Solihull Research Ethics Committee.

What if I change my mind?

You have the right to stop taking part in the study at any time. You can also withdraw data you have provided to us at any time, until the data have been submitted for publication. You will be given the contact details of the researcher if you wish to contact her. You will also be given a telephone number, which you can call and leave an answer machine message if you wish to withdraw from the study. There are no negative consequences of withdrawing from the study.

What if there is a problem?

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions using the details at the bottom of this form. If you remain unhappy and wish to complain formally, you can do this by contacting Research and Commercial Services at the University of Birmingham. Details can be obtained from the researcher or www.rcs.bham.ac.uk.

What happens when the study is finished?

After the study has finished, we will give you an information sheet about the study that describes sources of support for eating and parenting. If you wish to receive a copy of the study's findings, you will be able to do this once the study is completed by following links on the Infant and Child Laboratory website www.icl.bham.ac.uk.

What do I do if I want to take part?

All we ask of you on the maternity ward is for your consent for us to contact you in two days time to discuss whether you may be interested in taking part. If you think you are interested in this study, you can provide us with your contact details now and we will contact you in two days time, or you can telephone or email us using the information below.

Thank you very much for considering to take part in our research.

If you have any questions then please contact:

**Samantha Rogers,
School of Psychology,
University of Birmingham,
Edgbaston,
Birmingham,
B15 2TT**



Some Sources of Support

- Association for postnatal illness
Supports mothers suffering from postnatal illness
Website: www.apni.org
Telephone: 020 7386 0868
- Beat (beating eating disorders)
Provides information and support for carer's and sufferers of eating disorders
Website: b-eat.co.uk
Telephone: 0845 634 1414
- Citizen's Advice Bureau
Provides free, independent and confidential advice on legal and monetary issues
Website: www.citizensadvice.org.uk
Telephone: 0844 477 1010
- Cry-sis
Provides self-help and support to families with excessively crying, sleepless and demanding babies
Website: www.cry-sis.org.uk
Telephone: 0845 1228 669
- Depression Alliance
Provides help and information about depression
Website: www.depressionalliance.org
Telephone: 0845 123 2320
- Lone Parent Helpline
A free independent helpline for lone parents and anyone affected by the issues surrounding one-parent families
Website: www.loneparenthelpline.info
Telephone: 0808 802 0925
- Mind
Provides high-quality information and advice, and campaigning to promote and protect good mental health for everyone
Website: www.mind.org.uk
Telephone: 0845 766 0163
- Parent Line
Provides help and support to anyone caring for children and understands both big and small challenges
Website: www.parentlineplus.org.uk
Telephone: 0808 800 2222

- Patient Advice and Liaison Services (PALS)

Provides advice and support to patients, families and their carers, information on the NHS and health related matters and complaints procedures, assistance in resolving problems and concerns quickly

Website: www.pals.nhs.uk

Telephone: 0121 627 8820

- Samaritans

Provides confidential emotional support to those experiencing distress, despair and suicidal feelings

Website: www.samaritans.org

Telephone: 08457 90 90 90

- Women's Aid

Provides information and support to women experiencing domestic violence and/or sexual abuse

Website: www.womensaid.org.uk

Telephone: 0808 800 2222

APPENDIX 3

Consent for contact form



UNIVERSITY OF
BIRMINGHAM

Consent for Contact Form

Title of study: Breastfeeding, cortisol, infant growth and eating.

Name of Primary Researcher: Samantha Rogers

Please tick the boxes next to each statement and then sign and date the form below.

- I confirm that I have received and read the information sheet explaining the above study.
(Tick) (Initial)
- I agree to be contacted by the researcher in two days time to discuss whether I am interested in taking part in the study.
(Tick) (Initial)
- Please provide your contact details below.

Name:

Telephone number:

_____	_____	_____
Name of parent/carer	Date	Signature
_____	_____	_____
Name of researcher	Date	Signature

APPENDIX 4

Instructions for nappy collection



UNIVERSITY OF BIRMINGHAM

Instructions for nappy collection

Thank you for volunteering to take part in my study. When your baby is 1-week, 1-month, 3-months, 6-months and 12-months old, we would like to collect samples of used nappies from a 24-hour period. This will be so that we can measure the level of cortisol (a hormone involved in blood sugar regulation) in your infant's urine. We will visit you at each of these time points. Before each visit, please collect the samples of nappies using the following instructions.

We can only use nappies that contain fluid-absorbing granules such as Pampers, Huggies, Boots or similar. Unfortunately we cannot use eco nappies or cotton nappies.

Please use the same type of nappy throughout the collection day and avoid putting creams on your baby's bottom if possible.

Please inform the researcher of the brand name and the size (age group) of the nappies and provide a clean nappy of the same size and brand as used for the actual collection. This information will be used to determine the dry weight of the specific nappy.

We are unable to use nappies that are heavily contaminated with faeces ("poo"). We can only process nappies that contain urine ("wee"). Odd stains and spills of poo are not a problem but a completely soiled nappy cannot be used. Just discard such nappies.

If your baby passes a lot of poo or has diarrhoea the nappy collection should be postponed until one or two weeks later. Just let us know and we can easily re-arrange the visit.

We will provide you with a large Tupperware box with your participant details on it and the date when we intend to collect it (which we will arrange at your convenience). If you collect the sample of nappies a day or so before we visit, then if possible, please keep nappies in the freezer (-20 C) inside the Tupperware box.

Start collecting the nappies at a specific time of day that you will easily remember (e.g. 1st nappy of the morning). Then, put every nappy for the next 24 hours into the box until you reach the same time point the next day.

APPENDIX 5

Consent form



UNIVERSITY OF
BIRMINGHAM

Consent Form

Title of study: Breastfeeding, cortisol, infant growth and eating.

Name of Primary Researcher: Samantha Rogers

Please tick the boxes next to each statement and then sign and date the form below.

- I confirm that I have read and understood the information sheet for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.

(Tick) (Initial)
- I understand that my participation is voluntary and that I am free to withdraw my child and myself from the study at any time, without giving any reason.

(Tick) (Initial)
- I understand that the study involves five points of contact over the first year of my child's life and includes; the completion of questionnaires, measurement of the weight and length of my child, two observations of myself and my child during a mealtime, the provision of my own urine samples and donation of used nappies for assessment of cortisol.

(Tick) (Initial)
- I agree to my health visitor being informed of my participation in the study.

(Tick) (Initial)
- I agree to take part in the above study.

(Tick) (Initial)
- I agree to the audio and video recording of two observations of myself and my child during a mealtime.

(Tick) (Initial)

- I give permission for the samples I provide to be used in future ethically approved research

(Tick) (Initial)

Name of parent/carer Date Signature

Name of researcher Date Signature

APPENDIX 6

Consent form for further contact



UNIVERSITY OF
BIRMINGHAM

Consent Form for Further Contact

Title of study: Breastfeeding, cortisol, infant growth and eating.

Name of Primary Researcher: Samantha Rogers

Please tick the box next to the statement and then sign and date the form below.

- I agree to be contacted by the research team when my infant is 2-years-old. I give consent for my infant to be weighed, measured, and for a urine sample to be collected.

(Tick)

(Initial)

Name of parent/carer

Date

Signature

Name of researcher

Date

Signature

APPENDIX 7

Demographic and additional information sheet

Participant ID:



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Demographic and Additional Information

Previous research has demonstrated that demographic factors such as household income and educational attainment are related to different outcomes in this kind of study. Therefore please complete the following information about yourself and your newborn child.

Please complete the following information about your newborn

1. Gender:

2. Date of Birth:

3. At how many weeks of pregnancy or gestation was your child born (e.g. 38/39/40)?

4. Birth weight:

5. Length at birth:

6. Apgar score at birth:

Some of these details will be in your 'red book' from your health visitor, if you do not know them

7. Please describe your infant's ethnicity (Please circle)

White British

White Irish

Other White

Asian Indian

Asian Pakistani

Asian Bangladeshi

Other Asian

Black Caribbean

Black African

Other Black

Chinese

Mixed

Other (please state)

Please complete the following information about yourself

8. Age:

9. Pre-pregnancy weight:

10. Please state your partner's height and weight.

11. What is your ethnicity? (Please circle)

White British

White Irish

Other White

Asian Indian

Asian Pakistani

Asian Bangladeshi

Other Asian

Black Caribbean

Black African

Other Black

Chinese

Mixed

Other (please state)

12. Which category would best describe your total household weekly income (before tax)?

£150 or below

£151 - £200

£201 - £250

£251 - £300

£301 - £350

£351 or above

13. Is this income made up of mostly:

State benefits (such as Job Seekers allowance/income support)

Other benefits that subsidise wages (e.g. WFTC)

Maintenance payments for children

Wages

Other

14. What age did you (and your partner) leave school or finish your education?

	Yourself	Partner
Left school before 13 years old	<input type="checkbox"/>	<input type="checkbox"/>
Left school between 13 and 16 years	<input type="checkbox"/>	<input type="checkbox"/>
Further secondary education (16-18 years)	<input type="checkbox"/>	<input type="checkbox"/>
Secretarial/technical qualification	<input type="checkbox"/>	<input type="checkbox"/>
Teacher training	<input type="checkbox"/>	<input type="checkbox"/>
University course not completed	<input type="checkbox"/>	<input type="checkbox"/>
Professional qualification without degree	<input type="checkbox"/>	<input type="checkbox"/>
Degree	<input type="checkbox"/>	<input type="checkbox"/>
Further degree	<input type="checkbox"/>	<input type="checkbox"/>
NA	<input type="checkbox"/>	<input type="checkbox"/>
Not known	<input type="checkbox"/>	<input type="checkbox"/>
Other (please state)	<input type="checkbox"/>	<input type="checkbox"/>

15. How do you plan to feed your baby?

- Breastfeed
Formula feed

16. How many other children do you have? _____

Certain factors such as some medical problems and current medication can affect cortisol levels. Therefore the following questions will ask a little about your pregnancy and delivery.

17. Have you ever suffered from any of the following medical problems? Please tick the relevant boxes.

- Diabetes
High blood pressure
Low blood pressure
Asthma
Hyperthyroidism
Hypothyroidism
Epilepsy

18. Please describe any medications you are regularly taking and briefly what they are being taken for, e.g. oral contraceptives, inhalers etc.

19. Were there any complications during your pregnancy at all (for example, gestational diabetes, high blood pressure)?

Yes

No

20. If yes, what were the complications? (Please describe below)

21. Were the complications treated and if so, how? (Please describe below)

22. Please tick the box that best describes the birth of your baby.

Normal (vaginal) delivery

Caesarean

Forceps

Ventouse

23. Do you smoke?

Yes – go to question 24

No – go to question 26

24. How many cigarettes did you smoke during your pregnancy?

0-4 per week

5-20 per week

21-35 per week

36-70 per week

More than 70 per week

25. How many cigarettes do you currently smoke?

0-4 per week

5-20 per week

21-35 per week

36-70 per week

More than 70 per week

26. Do you currently drink alcohol?

Never

Once a month

About twice a month

Once a week

Two to three times per week

Four or more times per week

27. Please state the units of alcohol you consumed per week during your pregnancy

0 units per week

1-13 units per week

14-21 units per week

More than 21 units per week

28. Please state the units of alcohol you currently consume per week

0 units per week

1-13 units per week

14-21 units per week

More than 21 units per week

APPENDIX 8

Additional information sheet



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Additional Information

Previous studies have shown that certain factors such as current medication can affect cortisol levels. Therefore please complete the following information about yourself.

1. Current weight

2. Please describe any medications you are regularly taking and briefly what they are being taken for, e.g. oral contraceptives, inhalers etc.

3. Do you smoke?

Yes – go to question 4

No – go to question 5

4. How many cigarettes do you smoke?

a. 0-4 per week

b. 5-20 per week

c. 21-35 per week

d. 36-70 per week

e. More than 70 per week

5. Do you currently drink alcohol?
- a. Never
 - b. Once a month
 - c. About twice a month
 - d. Once a week
 - e. More than once a week
6. Please state the units of alcohol you consume per week
- a. 0 units per week
 - b. 1-13 units per week
 - c. 14-21 units per week
 - d. More than 21 units per week

APPENDIX 9

Feeding diary

Feeding Diary

Please complete this diary daily by stating...

- * The approximate number of feeds your baby has had each morning, afternoon, evening and night
- * How long each feed approximately lasted
- * The quantity taken (if formula feeding)
- * Whether the feed was breast or formula milk

	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Morning	Number of feeds:						
	Duration per feed:						
	Quantity taken:						
	Breast/formula:						
Afternoon	Number of feeds:						
	Duration per feed:						
	Quantity taken:						
	Breast/formula:						

	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Evening	Number of feeds: Duration per feed: Quantity taken: Breast/formula:						
Night	Number of feeds: Duration per feed: Quantity taken: Breast/formula:						

APPENDIX 10

Feeding information



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Feeding Information

1. Are you still breastfeeding your baby?

Yes – go to question 2

No – go to question 5

2. Are you feeding your baby just breast milk or have you introduced formula?

Just breast milk

Breast milk and formula

3. On average, how many times do you breastfeed your baby in 24 hours?

4. On average, how long does your baby breastfeed for per feed?

5. On average, how many times do you feed your baby using formula milk in 24 hours?

6. How much does your baby take per formula feed?

7. On average, how long does your baby feed per formula feed?

8. If you have stopped breastfeeding, after how many weeks/months did you stop?

9. Have you introduced solids to your child yet (e.g. baby rice)?

Yes

No

10. How old was your child when you first introduced solids?

11. What solids do you feed your child? (Please circle one of the answers below)

- a. Only home-made food
- b. Mostly home-made food
- c. About the same quantity of home-made and ready prepared food
- d. Mostly ready-prepared food
- e. Only ready-prepared food

APPENDIX 11

Edinburgh Postnatal Depression Scale (EPDS)



APPENDIX 12

Modified Infant Child Eating Behaviour Questionnaire (CEBQ)

Participant ID:

Infant CEBQ

Infants have a lot of different eating habits. Please rate how often your infant does the following things.

	Never	Seldom	Sometimes	Often	Always
1. My infant loves food					
2. My infant has a big appetite					
3. My infant finishes his/her food quickly					
4. My infant is interested in food					
5. My infant refuses new foods at first					
6. My infant eats slowly					
7. My infant enjoys tasting new foods					
8. If allowed, my infant would eat too much					
9. My infant enjoys a wide variety of foods					
10. My infant does not always eat all of the food in the bowl/plate					
11. My infant takes more than 30 minutes to finish a meal					
12. Given the choice, my infant would eat most of the time					
13. My infant gets full before his/her meal is finished					
14. My infant enjoys eating					
15. My infant is difficult to please with meals					
16. My infant gets full up easily					
17. Even if my infant is full up, s/he finds room to eat his/her favourite food					
18. If given the chance, my infant would drink continuously throughout the day					
19. My infant cannot eat a meal if s/he has had a snack just before					
20. If given the chance, my infant would always be having a drink					
21. My infant is interested in tasting food s/he hasn't tasted before					
22. If given the chance, my infant would always have food in his/her mouth					
23. My infant eats more and more slowly during the course of a meal					

APPENDIX 13

Nappy extraction instructions/Lab instructions for nappy extraction

Nappy Extraction Process

Additional Notes:

- We will be using 4% saline solution
- ** Scales:
 - o If the scales are wobbling on zero, press the 'T' button
 - o If the contents of the weighing boat are too heavy the scales will say 'H' – to solve this problem, weigh in two batches, making sure to minus the weight of both weighing boats from the total, and add the two together to get the sum.
- You should get roughly half of the solution back (after saline has been added)
- Taking nappies out of the freezer – make sure you only take the infants nappies out and leave the mother's urine sample in the freezer.

Stage 1:

- 1) Put paper towels down on the bench
- 2) Have an extra sheet of paper and a pen handy
 - a. Make a list of details to record during the experiment written on the paper including: the participant number, the date, how old the infant was when the sample was taken, how many nappies there are in the sample.
 - b. Also include: Individual nappy weight, total nappy weight, total gel weight, quantity of saline solution added, how much solution gained after gel filtration.
- 3) Put on vinyl gloves (white box)
- 4) Weigh each nappy individually and record its weight
- 5) Have at least two weighing boats available and record the weight of the weighing boats (in order to minus that weight from future total weights)
- 6) Open the nappies (dissect using scissors) and tear the top layer off
- 7) Extract the wet cotton from the inside of the nappy (dispose of the dry contents) and put it into a weighing boat
- 8) Wrap the remains in tissue and dispose (in a clear disposal bag)
- 9) Weigh the wet contents in the weighing boats (minus the weighing boat weight from the total). When carrying the weighing boat and its wet contents round to the scales, cover the contents with another weighing boat **
- 10) Write the participants number, age and date on a piece of tape (right of the counter) and attach to a beaker.
- 11) Put the nappies (all) in the beaker
- 12) Add 4% saline solution (1ml for every gram of gel) – shake saline, measure (measuring cylinder) and add the correct amount to the beaker. To test that enough liquid has been added, take a hand full of the solution and squeeze. If liquid comes out, there is enough solution.

- 13) Make a record of how much saline has been added
- 14) Work the solution until mixed.
- 15) Cover the beaker in foil (foil on the windowsill near the sink) and place a bit of towel roll under the beaker
- 16) Take to the cold room and place on a shelf for 24-hours

Stage 2 (24- to 48- hours later)

- 1) If you do not see any liquid, add more saline solution and put back in the cold room for another 24-hours. There is enough saline solution present if liquid comes out of the solution when it is squeezed. Add enough saline so that when the beaker contents are squeezed saline solution comes out.

- 2) If extra saline solution is added – record how much!

CHANGE SAMPLE, CHANGE GLOVE

- 3) Peel the label off the beaker and stick it to the table – to remind you of which sample you are looking at,

****If the nappy in question is a Pampers nappy, skip stage 5 and go straight to stage 7 and do this twice****

- 4) Pour the contents of the beaker into a potato ricer (held above another beaker) and squeeze until all of the liquid comes out. If there is a little bit of liquid on top of the squeezing device, turn it upside down and add to the solution in the new beaker.
- 5) Empty the contents of the potato ricer onto some tissue wrap it up and dispose of it in a clear disposal bag
- 6) Filter the contents of the new beaker into another beaker using a funnel and glass wool
- 7) Record approx how much solution you get back after filtration.
- 8) Label two tubes (located in the bottom right cupboard of the bench) with the following information:
 - a. Sam Rogers
 - b. Participant number
 - c. Visit (e.g. 6-months)
 - d. Baby or Mother (whose sample is it?)
- 9) Stir the solution and fill two tubes to about 13ml (the solution may expand slightly upon freezing)
- 10) Put the two tubes in the freezer, making sure they are positioned upright in the freezer. The freezer to use is the third freezer on the left, bottom draw.

11) Pour the excess solution down the sink.

Cleaning up

- 1) Lay out some more tissue
- 2) Wash out all equipment with tap water (no detergents needed), scrub around with a gloved hand
- 3) Rinse equipment with distilled water
- 4) Put all washed equipment upside down on the paper towels laid out earlier.
- 5) Cleaning the scissors: Squirt with ethanol and scrub with tissue.
- 6) Wipe the surface with ethanol.
- 7) Tie waste bag up with tape (but not too tight) and label with the lab number: 238.
- 8) Dispose of the bag in the bin in the refuse area.

APPENDIX 14

Lab records for extraction

Lab Records for Extractions

Lab records – record before lab work begins

Participant Number	
Date	
How old was the infant when the sample was taken (months)?	
Number of nappies in sample	

Lab records – to be collected during experimentation

Individual nappy weight								
Weighing boat weight for individual nappy								
Total nappy weight, minus weighing boat weight								
Gel weight								
Weighing boat weight used to weigh gel								
Total gel weight, minus weighing boat weight								
Quantity of 4% saline solution added to gel								
Quantity of solution gained back after 24 hours								

APPENDIX 15

GC/MS instructions

Steroid Extraction for GC/MS

(1) *Transfer 1ml urine from universal container to glass tube*

(2) *C18 Sep pak extraction (extracts steroids into methanol) (MeOH = methanol, dH₂O = distilled water)*

Wash sep pak cartridge with 4ml MeOH followed by 4ml dH₂O

Add sample to sep pak cartridge (wash glass tube out with small volume of dH₂O) and apply drop by drop

Wash with 4ml dH₂O – also applied drop by drop

Elute into clean glass tube with 4ml MeOH – waste first 3 drops

(3) *Evaporate methanol under N₂ at 55°C (N₂ = nitrogen)*

(4) *Hydrolysis (this removes the glucuronide and sulphate groups from conjugated steroids)*

Make up sample number + 1 times of the following hydrolysis mix:-

3ml 0.1M acetate buffer (20ml 1M acetic acid + 30ml 1M Na acetate + 450ml dH₂O – pH 4.8-5)

10mg ascorbate (Sigma)

10mg sulphatase (Sigma) (this enzyme also contains the glucuronidase)

Add 3ml of this mix to each tube, mix well (vortex) and heat at 55 °C for 3-hours

Cool tubes to room temperature

(5) *Sep pak extraction (extracts steroids back into methanol)*

Wash sep pak cartridge with 4ml MeOH followed by 4ml dH₂O

Add sample to column (wash glass tube out with small volume of dH₂O) and apply drop by drop

Wash with 4ml dH₂O – also applied drop by drop

Elute into clean glass tube (silylated) with 4ml MeOH – waste first 3-4 drops

(6) *Add 100µl internal standard to each sample with glass syringe*

(Internal standard is a mix of the synthetic steroids stigmaterol and cholesterol butyrate which we use to quantify the steroids)

(7) *Evaporate methanol under N₂ at 55°C*

(8) *Derivitization*

Add 3 drops of 2% methoxyamine-pyridine and vortex tubes

Heat at 55 °C for 1-hour

Evaporate under N₂ at 55°C

Add 50µl TMSi per sample, vortex and put tubes in oven (120°C) overnight

(9) *Extraction of steroids into cyclohexane for injection*

Add 2ml cyclohexane to each sample and vortex

Add 2ml dH₂O to each sample and vortex

Centrifuge tubes at 1600rpm for 5 mins

Remove bottom layer (dH₂O) into waste

Add a further 2ml dH₂O, vortex and centrifuge as above

Transfer top layer (cyclohexane) to clean glass tube (silylated)

Evaporate under N₂ at 55°C

Add 350µl cyclohexane, vortex and transfer to injection vials

(10) *Injection of samples*

Inject samples into GC/MS along with the external standard

The external standard contains known quantities of all the steroids to be measured and is used to calibrate the machine each time a batch of samples is run. The results for the samples are then calculated by comparing the areas of each steroid peak in the external standard with those in the sample.

APPENDIX 16

Differences between those who did, and did not, withdraw

T-tests exploring demographic differences in participants who did, and did not, withdraw from the study

	t	df	p
Household income	-1.95	79	.06
Number of other children	1.37	79	.17
Alcohol consumption during pregnancy	1.37	79	.11
Alcohol consumption at 1-week	-0.18	79	.86
Alcohol consumption at 1-month	.43	75	.67
Alcohol consumption at 3-months	1.56	72	.12
Alcohol consumption at 6-months	1.04	71	.38
EPDS score at 1-month	-0.13	75	.90
EDPS score at 6-months	0.73	71	.47
Infant birth weight SDS	-0.82	79	.40

APPENDIX 17

Differences between those who were, and were not, observed

T-tests exploring demographic differences in mothers who were, and were not, observed feeding their infants at 6-months

	t	df	p
Maternal age	0.94	71	.35
Household income	1.41	71	.16
Education	1.83	71	.07
Number of other children	0.55	71	.58
Number of cigarettes smoked per week	-0.35	71	.73
EPDS score at 6-months	-1.05	71	.30
6-month infant weight SDS	0.02	71	.98

T-tests exploring demographic differences in mothers who were, and were not, observed feeding their infants at 12-months

	t	df	p
Maternal age	1.12	67	.28
Household income	1.29	67	.22
Number of other children	0.14	67	.89
Number of cigarettes smoked per week	-1.46	67	.07
Units of alcohol consumed per week	1.90	67	.06
EPDS score at 12-months	-0.10	67	.92
12-month infant weight SDS	0.88	67	.38

APPENDIX 18

Samples provided at each time point

Total number of mothers who provided urine samples at each time point of the study

	1-week	1-month	3-months	6-months	12-months
N agreed to provide samples	<i>mother</i>	<i>mother</i>	<i>mother</i>	<i>mother</i>	<i>mother</i>
59	45	53	46	48	41

Total number of infants who provided urine samples at each time point of the study

	1-week	1-month	3-months	6-months	12-months
N agreed to provide samples	<i>infant</i>	<i>infant</i>	<i>infant</i>	<i>infant</i>	<i>infant</i>
59	50	56	56	50	48

APPENDIX 19

Intraclass correlation coefficients demonstrating inter-rater reliability in observations of infant feeding

Intraclass correlation coefficients between researcher and research assistant codings of observed mealtimes

	Vocalisations	Appropriateness	Sensitivity
6-months	.96	.84	.77
<i>n</i>	58	58	58
12-months	.99	.91	.91
<i>n</i>	55	55	55