Biology and Environment, Mothers and Infants: Linking Stress Physiology, Depression, Anxiety and Attachment

Judith Catherine Warner
BPsych (Hons) Griff. 

School of Applied Psychology
Griffith Health
Griffith University, Mount Gravatt

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Abstract

The last two decades have seen research on early life experiences expand to include the prenatal environment and, more specifically, examination of the effects of prenatal maternal mental health on foetal and infant development. Cortisol, the hormonal end product of the Hypothalamic Pituitary Adrenal (HPA) Axis, has been identified as one mechanism associated independently with stress, pregnancy and mental health, which can account for changes during foetal development. In the postpartum, these early life experiences may serve as protective or risk factors for the infant. The longitudinal study conducted here examined nulliparous pregnant women \((N = 40, M_{\text{age}} = 30.5, SD = 5.27)\) and their infants commencing during the first trimester of pregnancy until 12 months after birth, with the aim of identifying potentially modifiable mother-infant characteristics associated with mothers' mental health, infant stress physiology and attachment.

The longitudinal study was subdivided into four studies. First, in Study 1A, concurrent and prospective associations between maternal stress (cortisol and self-report of daily stress), coping, and mental health were examined across the three trimesters of pregnancy. Mothers completed questionnaires and gave saliva samples during each trimester of pregnancy and overall the findings showed the important role of coping in modulating baseline cortisol levels and anxiety in the face of daily stressors during pregnancy.

Second, in Study 1B, the importance of early identification of prenatal risk factors for postnatal depression (PND) to preserve mother and infant wellbeing was recognised and investigated. It was found that PND symptoms were elevated in women who reported more general anxiety, more pregnancy-related anxiety, more stress in the second trimester, and more anxiety and stress in the third trimester of pregnancy.
Mothers who had higher cortisol and anxiety about their child’s health, relative to other mothers, across the three trimesters had more symptoms of PND when measured two months after the child's birth. Also, a path model showed that anxiety fully mediated associations of prenatal maternal basal cortisol, depressive symptoms and stress with PND symptoms.

Third, in Study 1C, maternal cortisol and perceived stress during pregnancy were linked to infant baseline cortisol and cortisol reactivity. Measures of mothers’ prenatal baseline cortisol levels, daily stress, and mental health symptoms, as well as mental health symptoms two and four months after birth, were assessed. Infant saliva samples were collected before and after 2-month and 4-month vaccinations to assess baseline cortisol and to determine cortisol reactivity and recovery. Lending support to the foetal programming hypothesis, mothers’ higher cortisol levels and greater perceived daily stress during pregnancy, were associated with infants’ higher cortisol at baseline, but lower response and greater recovery from stress at four months, perhaps indicating a priming effect whereby the system was primed for stress but regulated efficiently. However, at four months of age a moderation effect was found, whereby infants of mothers who had reported more mental health problems two months prior showed greater decline in cortisol recovery from 2 to 4 months of age.

Finally, in Study 1D, relationships were examined between maternal prenatal cortisol, mental health in the pre- and post-natal periods, infant attachment behaviour, and other responses during the Strange Situation. Prenatal maternal basal cortisol and anxiety were associated with infant baseline cortisol at twelve months of age. Maternal anxiety and depression symptoms reported in the postpartum period (when infants were 2, 4, and 12 months of age) were strongly negatively associated with infant cortisol recovery from the Strange Situation conducted when they were twelve months of age.
When maternal anxiety and depression were higher, infant cortisol recovery from the stressor was poorer. Additionally, mothers' mental health symptom level, reported when their infants were twelve months of age, was associated with higher infant baseline cortisol levels and poorer cortisol recovery from stress. There were no significant differences in cortisol levels between infants with secure compared to insecure attachment classifications. However, infants’ cortisol recovery was lower when mothers’ mental health symptoms were high and infants used more proximity seeking.

The current study findings suggest that: (1) prenatal maternal cortisol level, psychological stress, coping, and mental health symptoms are linked during pregnancy, and coping can reduce the association between mothers' perceived stress and their cortisol level, (2) there may be a pathway whereby cortisol and perceived stress during pregnancy increase mothers' general anxiety, which in turn places mothers at greater risk of PND symptoms, (3) maternal cortisol and anxiety during pregnancy are associated with 2-month-old infants' baseline cortisol levels, reactivity and recovery, and (4) when mothers' report more symptoms of depression and anxiety in the postnatal period, these are even more strongly and consistently associated with infants' baseline cortisol and recovery than are measures taken during pregnancy.
Statement of Originality

This work has not previously been submitted for a degree or diploma in any university. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made in the thesis itself.

(Signed)______________________________

Judith Catherine Warner
Publications

Publications


Poster Presentation

Mothers and Infants

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CHAPTER 1

Introduction

In the last decade, developmental psychologists have identified multiple prenatal and early life experiences that have the potential to explain typical and atypical developmental patterns beginning in infancy and continuing throughout the lifespan. Prior to birth, some of these experiences include exposure to maternal stress (e.g., marital discord), maternal depression and anxiety and, more specifically, pregnancy-related anxiety (Diego, Field, Hernandez-Reif, Cullen, Schanberg & Kuhn, 2004; Huizink, Robles de Medina, Mulder, Visser & Buitelaar, 2003; Talge, et al., 2007; Van den Bergh, Van Calster, Smits, Van Huffel & Lagae, 2008). In the early years, after birth experiences associated with infants’ social, emotional and behavioural developmental pathways include stress in the family, caregiver mental health, parenting behaviours, and the early attachment relationship between a young child and a caregiver (Bugental, Martorell & Barraza, 2003; Diego et al., 2004; Radke-Yarrow, 1991). Some of the outcomes linked with prenatal stress include preterm birth (de Weerth, van Hees & Buitelaar, 2003; Killingsworth Rini, Wadhwa, Dunkel-Schetter & Sandman, 1999), impaired cognitive and motor development (Buitelaar, Huizink, Mulder, Robles de Medina & Visser, 2003; Huizink et al., 2003), difficult temperament (Davis, Glynn, Schetter, Hobel, Chicz-Demet & Sandman, 2007; de Weerth et al., 2003; Huizink et al., 2003) and behavioural and emotional problems (O'Connor, Heron, Golding, Beveridge & Glover, 2002). Some of the outcomes linked with postnatal caregiver stress, mental health symptoms, such as postnatal depression (PND), and parenting behaviours include insecure attachment behaviour, impaired emotional regulation, impaired cognitive abilities and sad and anxious behaviour in infants (Milgrom, Westley & Gemmill, 2004; Radke-Yarrow, 1991).
Some theorists argue that the associations between prenatal negative experiences and children’s development can be partially accounted for by physiological mechanisms. Researchers have hypothesised that insults suffered by the foetus during critical developmental periods cause responsive changes across structural, hormonal and metabolic systems, an occurrence known as foetal programming (Barker, 1998; Barker, 2002). It is thought that foetal programming occurs during periods of rapid cell division when the developing organism is malleable and particularly vulnerable to environmental influences. It has been argued that the changes exacted on the developing foetus remain after the threat has passed and are the origins of some pathology in later life (Barker, 1998; Barker, 2004). For example, researchers have suggested that suboptimal prenatal and early postnatal environments can permanently alter the way an infant physiologically responds to stress, resulting in an overly elevated physiological response to stress and a reduced ability to regulate the stress axis when activated (Diego, Field & Hernandez-Reif, 2005; Diego et al., 2004; Radke-Yarrow, 1991). These difficulties with stress reactivity and regulation, in turn, may form the foundation for the development of behavioural and emotional problems. Empirical findings have supported these views. In humans and non-human primates, maternal stress, including mental health symptoms, during pregnancy has been associated with increased basal levels of infants’ cortisol, a biological marker of stress, and increased cortisol reactivity to psychosocial and physical stress (Brennan et al., 2008; Clarke, Wittwer, Abbott & Schneider, 1994; Davis, Glynn, Waffarn & Sandman, 2011; Field et al., 2010; O’Connor et al., 2005; Tollenaar, Beijers, Jansen, Riksen-Walraven, & De Weerth, C., 2011; Wadhwa, Dunkel-Schetter, Chicz-DeMet, Porto & Sandman, 1996). Further, suboptimal parenting behaviours such as corporal punishment and emotional withdrawal, and parental behaviours associated with insecure and disorganised
attachment classifications such as maternal depression (Bugental et al., 2003) and intrusiveness (Nachmias, Gunnar, Mangelsdorf, Parritz & Buss, 1996) have all been associated with greater cortisol reactivity in infants.

Cortisol is an important measure of stress in these studies as atypical basal levels of cortisol have been linked to poor child mental health outcomes in children as young as four and a half (Essex, Klein, Cho & Kalin, 2002). Studying even earlier years of the lifespan, Essex and colleagues found that basal cortisol levels in infants and maternal stress each made unique contributions to adverse child mental health in later life. Higher basal levels of cortisol have also been observed in maltreated children (aged 6 to 11) with clinical levels of internalising problems, such as depression and anxiety when compared to maltreated and non-maltreated children without internalising problems (Cicchetti & Rogosch, 2001), and in children (mean age 10.7 years) with post-traumatic stress disorder (PTSD) compared to age-matched children without PTSD. Furthermore, cortisol levels were significantly higher for girls with PTSD compared to boys with PTSD (Carrion et al., 2002). Lower basal levels of cortisol have been observed in boys (aged 8 to 11) with oppositional defiant disorder (ODD) when compared to boys without ODD (Van Goozen et al., 1998), maltreated children (aged 6 to 11) with clinical levels of externalising behaviour when compared to maltreated and non-maltreated children without externalising behaviour (Cicchetti & Rogosch, 2001), peer rejected children (aged 3 to 5) when compared to their non-rejected peers (Gunnar, Sebanc, Tout, Donzella & Van Dulmen, 2003) and children (aged 20 to 60 months) in foster care when compared to children who were not in foster care (Dozier et al., 2006).

Expanding this research to determine the point at which these discrepancies are first noted, research has focussed on infant cortisol levels and, more specifically, the relationship between maternal prenatal mental health symptoms and infant cortisol after
birth. Higher baseline cortisol levels have been observed in newborns of depressed women and those with comorbid anxiety and depression, compared to women with anxiety only and without depression (Field et al., 2010) as well as mothers with a lifetime history of depression (Tollenaar et al., 2011). Infant cortisol reactivity to stressors have been predicted by mothers’ pregnancy-related anxiety (Tollenaar et al., 2011) and comorbid depression and anxiety (Brennan et al., 2008). However, in one study of depressed women, higher quality mother-infant interaction actually predicted lower infant cortisol levels, indicating that the mother-infant relationship may potentially modify the effects of maternal mental health on infant cortisol (Letourneau, Watson, Duffett-Leger, Hegadoren & Tryphonopoulos, 2011). Combined, these studies suggest that maternal mental health stresses the infant and impacts on the infant Hypothalamic Pituitary Adrenocortical (HPA) axis.

**Overview of the Current Study**

The general purpose of the longitudinal study reported here was to examine concurrent and prospective associations between 1) prenatal maternal cortisol levels commencing in the first trimester of pregnancy, 2) prenatal maternal stress, depression, anxiety symptoms, report of pregnancy-related anxiety, and coping, 3) postnatal maternal stress, depression and anxiety symptoms, 4) infant baseline cortisol and cortisol reactivity to, and recovery from, particular stressors and 5) the mother-infant attachment relationship. This study was designed to extend on previous research in four ways. First, the longitudinal prospective design aimed to reduce reporting biases associated with retrospective designs, mental health problems and significant life events. Secondly, by commencing data collection in the first trimester of pregnancy and measuring key maternal variables throughout each trimester of pregnancy, to improve methodological design of previous studies and examine the role of maternal stress on
the developing foetus prior to development of the foetal HPA axis. Thirdly, by expanding on previous definitions of prenatal stress to develop a multidimensional conceptualisation and operational definition; and finally, by examining the associations of prenatal data and the concurrent development of infant physiological stress responses and attachment style throughout the first year of life.

Methodological designs of previous studies have been limited by retrospective design or by maternal cortisol being measured in the second and third trimesters of pregnancy. However, studies of humans and non-human primates indicate that maternal stress in early pregnancy has the greatest impact on infant behaviour (e.g., Davis et al., 2011; Schneider, Roughton, Koehler & Lubach, 1999). Subsequently, the importance of developmental timing of maternal stress and glucocorticoid exposure has been emphasised by previous researchers (Huizink et al., 2003). This will be addressed in the current study, examining maternal stress beginning in the first trimester of pregnancy, a period of rapid cell division and organ development when the foetus is particularly susceptible to environmental experiences and damage (Barker, 1998), the second trimester when the foetal HPA axis develops (Keller-Wood & Wood, 2001), and the third trimester when maternal cognitive appraisals of stress may be attenuated (Glynn, Dunkel-Schetter, Wadhwa & Sandman, 2004). After birth, maternal mental health symptoms and infants’ baseline cortisol and responses to, and recovery from, stress will be repeatedly examined during the first year of life. Finally, the quality of the mother-infant attachment relationship and infant emergent coping will be examined at twelve months of age.

**Overview of the Thesis Chapters**

In the following six chapters, a broad literature review is provided in Chapter 2, which is followed by four studies focussing on more specific background literature to
support the study aims and analyses of the data. These aims and analyses are presented in four studies reported in separate chapters (Chapters 3 to 6). Following these chapters, the final chapter (Chapter 7) includes a general discussion of the findings and describes future directions and implications for theory, research and clinical practice.

The background and literature review in Chapter 2 are focused on summarising major research on prenatal stress, maternal mental health and coping and caregiver-infant attachment. Additionally, this chapter highlights methodological weaknesses of previous research, proposes a multidimensional definition of prenatal stress and provides a rationale for the subsequent studies.

The following two chapters (Chapters 3 and 4) present the results of two studies that had the general aim of understanding maternal stress and mental health and their implications for maternal cortisol levels and postnatal depressive symptoms. Specifically the aim of the first study (Study 1A, Chapter 3) was to examine how stressful life events, mental health and maternal cortisol covary. More specifically, in Chapter 3, a review of prenatal stress and cortisol throughout pregnancy, maternal mental health, and coping is provided. Relationships were examined between maternal depression, stress, general and pregnancy-related anxiety, coping and cortisol throughout pregnancy commencing in the first trimester. The role of coping as a moderator between maternal mental health symptoms and cortisol was also examined.

To extend on the first study, the focus was again on maternal mental health in Study 1B (Chapter 4), but the key outcome was mothers' postnatal depressive symptoms. Correlations, regression, and growth curve modelling were used to examine patterns of maternal depression, stress, general and pregnancy-related anxiety and cortisol throughout pregnancy, and their associations with maternal depressive symptoms in the postpartum period (i.e., two months postbirth).
The next two studies reported in Chapters 5 and 6 (Studies 1C and 1D) shift the focus to infant outcomes. In the third study (Study 1C), the aim was to explain infants’ basal cortisol levels, cortisol reactivity to a stressor, and cortisol recovery after a stressor at two and four months of age by focusing on their associations with maternal prenatal cortisol levels and maternal life stress, depression and anxiety during the three trimesters of pregnancy. Maternal mental health symptoms in the postpartum period were also examined as potential moderators of infant cortisol reactivity to, and recovery from, a vaccination stressor between two and four months of age.

The fourth study reported in Chapter 6 (Study 1D) focused on predicting the mother-infant attachment relationship at 12-months of age, using Ainsworth and Wittig’s (1969) Strange Situation observational paradigm. An additional aspect of this study was to examine infants’ cortisol reactivity and recovery in response to the Strange Situation stressor and examine infant’s displays of coping behaviours ("emerging coping strategies") during the Strange Situation. Associations between these variables and maternal mental health symptoms throughout the first year were also examined.

Finally, Chapter 7 comprises a general discussion of the main findings from the four studies. Limitations of the studies are addressed and implications for mother and infant well-being, future research and clinical practice are also discussed.
Seminal work in the area of psychological stress and coping has suggested that stress is a result of environmental events that exceed coping ability (Lazarus & Folkman, 1994). However, more recently psychological stress has been defined as any event that increases the secretion of glucocorticoids (Heuser & Lammers, 2003). Cortisol, the hormonal end-product of the Hypothalamic Pituitary Adrenocortical (HPA) axis in primates, is widely used as a marker of stress in human and non-human primate research (e.g., Davis & Sandman, 2010; Davis, Glynn, Waffarn & Sandman, 2011; Feng et al., 2011; Gunnar, Talge, & Herrera, 2009). Cortisol is present in the body in low levels during non-stress periods (basal level) and at higher levels in response to stressors. Whereas cortisol reactivity to stress is adaptive in the short term, ongoing activation of the HPA axis is associated with physical and psychological impairment and neuronal death resulting in a system primed for stress and unable to regulate (recover from) the stress response when activated (Gunnar & Cheatham, 2003; Gunnar & Vazquez, 2006).

Maternal Stress and Mental Health, and Infant HPA Functioning

Cortisol and pregnancy. Cortisol levels increase throughout pregnancy, peaking between two and four times non-pregnancy levels between 26-34 weeks of gestation resulting in a clearly defined period of hypercortisolism (Mastorakos & Ilias, 2003; Sandman et al., 2006). During this time foetal cortisol levels are partially contingent on maternal cortisol with up to 40% of maternal cortisol passing through the placenta into foetal circulation (Gitau, Cameron, Fisk & Glover, 1998). Although some maternal cortisol is metabolised by the placenta (of foetal origin) theorists suggest it has the ability to influence the developing foetus prior to development of the infant HPA
axis (Nader, 2004). Structural changes may occur as a result of cortisol passing through the blood-brain barrier and affecting the developing nervous system (Davis & Sandman, 2012).

Around the second trimester of pregnancy, as the infant HPA axis matures, transportation of maternal cortisol reduces and the infant cortisol secretion increases (Keller-Wood & Wood, 2001). Research has indicated that shortly thereafter, towards the end of the second trimester, the foetal HPA axis exhibits stress responses independently of maternal responses (Gitau, Fisk, Teixeira, Cameron & Glover, 2001).

In human adults and animals glucocorticoids demonstrate a circadian rhythm whereby levels are typically highest in the morning on awakening and lowest in the evening prior to onset of sleep (Sapolsky, 1992). In contrast, infants exhibit two peaks of cortisol approximately 12 hours apart (Watamura, Donzella, Kertes & Gunnar, 2004). Researchers have suggested that diurnal rhythmicity typically evidenced in adults is established sometime between three (Larson, White, Cochran, Donzella & Gunnar, 1998) and six months of age (Lewis & Ramsay, 1995). Hence, it appears that a consistent pattern of cortisol secretion is established during the first half year of life (Lewis & Ramsay, 1995). A recent review examining cortisol reactivity in infants (aged 0 – 24 months) indicated physical, rather than psychological, stressors more reliably produced increases in cortisol. However, the authors reported the effect size of cortisol reactivity in response to physical stressors decreased with age with little being known regarding these patterns for infants over the age of six months (Jansen, Beijers, Riksen-Walraven & de Weerth, 2010). Nonetheless, in another review Gunnar and colleagues (2009) reported elevations in cortisol have been noted in infants (aged 9 – 18 months) in response to separations from caregivers and this was particularly true when alternative caregivers were low in responsiveness or when children had insecure attachment styles.
Maternal daily psychosocial stress, cortisol and pregnancy. Mothers’ environmental stress has been associated with higher basal levels of cortisol in their children. For example, maternal stress, measured by questionnaires and salivary cortisol during the second trimester of pregnancy, have each been linked to higher basal levels of cortisol in children aged 4 to 6 during stressful situations (Gutteling, De Weerth & Buitelaar, 2004; Gutteling, De Weerth & Buitelaar, 2005) suggesting that prenatal maternal stress may have permanently altered children's physiological stress responses. Extending this argument, maternal cortisol, measured early in the second trimester (i.e., 15 weeks gestation), has been associated with larger amygdala volume, an area of the brain associated with memory and emotional responses, and higher incidence of affective problems in girls (Buss et al., 2012). Evolutionary theory has often been drawn upon to explain these findings. For example, researchers have highlighted how animals are primed to attend to stressors in the environment thereby increasing their chances for survival (Matthews, 2002).

Previous findings from non-human primate studies suggest that infants who were exposed to prenatal maternal stress have a reduced ability to regulate the stress system when activated (Clarke, Wittwer, Abbott & Schneider, 1994). One mechanism through which the developing stress system may be altered is through an imbalance in mineralocorticoid (MR) and glucocorticoid receptors (GR) in the brain. The dysregulation of the HPA axis caused by this imbalance, in combination with impaired emotional, cognitive and behavioural responses, is thought to be linked to subsequent susceptibility to disease and psychopathology in later life (de Kloet & Oitzl, 2003; Gunnar & Quevedo, 2007; Sapolsky, 1992). However, it has been suggested that under conditions of optimal care (e.g., warm, sensitive caregiving), a human infant’s stress system enters a period of hypo-responsivity by the end of the first year that may protect the
developing brain (Gunnar, 2006). This likely indicates that the caregiver-infant relationship plays an important role in moderating the effects of prenatal maternal stress.

Research examining the relationship between stress and cortisol in humans has become more readily accessible since the development of salivary cortisol analysis. Cortisol is found in all body fluids and its presence in saliva is considered to be a reliable and valid reflection of unbound plasma levels of the hormone (Gunnar et al., 2009; Kirschbaum & Hellhammer, 1994; Kirschbaum, Strasburger, Jammers & Hellhammer, 1989). Prior to these advances in analyses the invasive nature of examining plasma levels of cortisol meant that most research took place in animal models.

Research on prenatal maternal stress and mental health, and infant cortisol. There are a number of studies that show prenatal psychosocial stress has a negative impact on infant psychological, cognitive and social functioning. In a seminal study Huizink and colleagues (2003) extrapolated from primate studies to describe how human prenatal maternal psychosocial stress can have a negative effect on infant mental health outcomes. Specifically, they suggested that a stressful prenatal environment may be linked to anxiety, depression, impaired cognition, learning and motor development in infants, as well as to less social interaction and shorter attention spans (Huizink, Robles de Medina, Mulder, Visser & Buitelaar, 2003). Similarly, Talge et al., (2007) suggested that infants are “substantially more likely to have emotional or cognitive problems” (p. 245) as well as being vulnerable to depression, anxiety and attention deficit disorders if they were exposed to prenatal maternal stress.

In the last decade the negative impact of prenatal maternal stress has been extended to try and understand physiological mechanisms that account for the
psychological, cognitive and social deficits in infants. Some of this research has focused on studying maternal cortisol and its impact on infant HPA axis functioning. For example, one recent study found that prenatal maternal cortisol and psychosocial stress each contribute to the development of infant difficulties (Davis et al., 2011). Examining infant cortisol and behavioural response to the heel-stick procedure within the first 24 hours after birth, researchers found that higher concentrations of maternal cortisol during late second and third trimester of pregnancy were associated with higher infant cortisol responses to the procedure. Further, maternal cortisol early in pregnancy (13-14 gestational weeks), and psychological stress throughout pregnancy, predicted slower rates of infants’ behavioural recovery from the stressor. The authors suggested postnatal factors were unlikely to account for these differences because infant cortisol was measured so early in life. Moreover, in a prospective longitudinal study that examined the effects of prenatal stress on child cortisol levels, researchers found that maternal self-report and physiological measures of stress during pregnancy were positively associated with child cortisol levels on their first day of school (Gutteing et al., 2005). Specifically, children of mothers who had higher cortisol levels at sixteen weeks gestation had higher levels of cortisol on their first day of school compared to children whose mothers had lower levels of cortisol. Further, the authors reported that pregnancy-related anxiety was positively correlated with children’s cortisol levels.

Other researchers have examined how prenatal maternal mental health may impact upon infants’ stress level and reactivity by measuring cortisol. In one study Field and colleagues (2010) reported that women who reported depression or comorbid depression and anxiety during pregnancy had newborns with higher basal levels of cortisol than those with anxiety alone or who were not depressed (Field et al., 2010). Other researchers reported pregnancy-related anxiety, but not cortisol or stress, during
the last trimester of pregnancy, was associated with greater infant cortisol reactivity to stressors at five weeks of age, but lower levels of cortisol reactivity to stressors at 2 and 12 months of age (Tollenaar, Jansen, Riksen-Walraven & De Weerth, 2011).

These recent prospective studies of human mothers and infants add substantially to the research in non-human primates and suggest prenatal maternal stress in humans is an important correlate of infant cortisol reactivity and related behavioural and emotional functioning. Further, these findings offer preliminary data to suggest that the effects of maternal stress and mental health symptoms on the foetus continue well into the first year of life and can even extend to later childhood. These effects appear to be most problematic when maternal mental health problems are ongoing. Findings also show that maternal cortisol during pregnancy is implicated in this process. Together, these stress and coping processes culminate in an established caregiver-infant attachment relationship by the end of an infant’s first year of life.

Maternal depression during pregnancy has also been associated with infant behaviours shortly after birth. In one study, infants of mothers who were either depressed or not depressed during mid-pregnancy were tested shortly after birth (Diego, Field, Hernandez-Reif, 2005). The infants born to mothers who had been depressed exhibited more stress behaviours, crying and indeterminate sleep (a marker for biobehavioural organisation) than those born to non-depressed mothers. Of note, higher basal levels of maternal cortisol have been found in depressed compared to non-depressed mothers, suggesting that it is possible that maternal cortisol could have accounted for these associations (Lundy et al., 1999). Further, Diego and colleagues (2004) found that maternal depression impacted on newborn physiological functioning and importantly, the onset and duration of maternal depression affected newborns’ physiology differentially. In this prospective study, 80 mothers were assessed for
depression between 23 and 27 weeks gestation, and again postpartum, in a university hospital. Mothers were classified as depressed during pregnancy (prepartum), depressed after pregnancy (postpartum), depressed during both periods (prepartum-postpartum), and not depressed. Analysis of urinary cortisol samples showed that depressed mothers had higher cortisol levels than non-depressed mothers. Further, while infants of depressed mothers showed a trend towards higher cortisol, this relationship was significant only for infants whose mothers were depressed during both the prepartum and postpartum periods (Diego, Field, Hernandez-Reif, Cullen, Schanberg & Kuhn, 2004). These findings suggest a link between prenatal mental health symptoms and human infant HPA activity, as well as illustrating the potential importance of the developmental timing of stressors during the entire perinatal period and the caregiver-infant relationship in the postnatal period.

**Research on prenatal maternal stress in non-human primates.** Animal models allow standardisation of psychosocial stress and experimental control not afforded in human studies. In support of the foetal programming hypothesis (Barker, 1998; Barker, 2002), the long term effects of prenatal stress on the infant HPA axis are suggestive that prenatal maternal stress affects the physiology and behaviour of non-human primates. Clarke and colleagues (1994) exposed 11 pregnant rhesus monkeys to unpredictable noise during mid to late pregnancy. Post parturition, blood samples were collected from infant monkeys on four occasions. Compared to controls, infants of stressed mothers had higher basal cortisol values. The juvenile rhesus monkeys were followed up at 18 months and blood samples were taken following four progressively more stressful challenges. Results showed that, compared to controls, infants of stressed mothers had significantly higher cortisol responses across all stress conditions. These findings indicate that stress during pregnancy can affect basal levels of cortisol as well
as reactivity to stressors later in life. Another study found that when a stress response was chemically induced in pregnant rhesus monkeys over a 2-week period, activation of the HPA axis was associated with infants who exhibited poor motor and attention ability, and increased irritability and inconsolability compared to controls at two weeks of age (Schneider, Coe & Lubach, 1992).

Timing of prenatal stress also appears to be important. In one study, two groups of pregnant rhesus monkeys were subjected to transportation and a noise stressor designed to activate the HPA axis. One group of mothers was stressed early in pregnancy, one group was stressed late in pregnancy and the control group remained undisturbed throughout pregnancy. Twenty-eight rhesus monkey infants, born to the three groups of mothers, were assessed for birth weight and neurobehavioral development. Compared to mid-late gestational stress and controls, those infants born to the early gestational stress group weighed less at birth. Furthermore, whereas infants born to the stressed mothers exhibited attention and motor deficits compared to controls, these impairments were most marked in the early gestation stress group. Moreover, during the first month of life, development of the control group increased linearly, however, this trend was not evident in either of the stress groups (Schneider, Roughton, Koehler & Lubach, 1999).

**Maternal Stress and Mental Health, and Infant Attachment**

**Caregiver-infant attachment and Attachment Theory.** Infants are very dependent upon caregivers for soothing when they are distressed (Skinner & Zimmer-Gembeck, 2007; Sroufe, 1996). Research has indicated that infants who cope with stress by seeking proximity to their caregivers are less likely to exhibit physiological stress responses than infants who show less adaptive coping behaviours, such as anger or avoidance (Hertsgaard, Gunnar, Erickson & Nachmias, 1995). One important factor
associated with the development of infant coping and cortisol reactivity is the caregiver-infant relationship. Understanding the implications of the relationships between these variables are burgeoning areas of research in developmental psychology and developmental neuroscience. The caregiver-infant relationship has been examined from psychoanalytic (e.g., Freud) and social learning (e.g., Beller) theoretical backgrounds, suggesting that the caregiver-infant bond develops through drive or dependency (Ainsworth, 1969). However, the current longitudinal study was founded on the ethologically based classic Attachment Theory developed by John Bowlby (1969) and Mary Ainsworth (1969). Encompassing ethology and developmental psychology, this theory may be best understood within the context of evolution. The central tenet of Attachment Theory is that the human infant maintains proximity to the caregiver, through behavioural mechanisms, in order to reduce vulnerability throughout infancy and childhood (Ainsworth, 1969; Bowlby, 1969). Therefore, under ideal circumstances, attachment behaviour is activated during times of stress and promotes close proximity to the caregiver for comfort and safety, and when this relationship is optimum, stress is reduced by the close physical proximity to the caregiver.

**Attachment Theory.** In Attachment Theory it is argued that attachment patterns between a caregiver and an infant develop during the first year of life and are best assessed between 12 and 24 months of age (Ainsworth, Blehar, Waters & Wall, 1978). This attachment pattern has been described as an outcome of a history of caregiver-infant interactions during an infant's first year. During this first year the infant draws from previous experiences and outcomes to develop a representation of the caregiver and her/his likely behaviour (Bowlby, 1969), and organises his or her own behaviour around these particular expectations and outcomes (Ainsworth, 1969; Sroufe & Waters, 1977). From the foundation of these interactions, the infant first learns how to cope with
stress by relying on the security provided by proximity to a caregiver (Bowlby, 1951), but subsequently develops self-regulatory capacities that supplement the use of others for support (Bowlby, 1951; Carlson & Sroufe, 1995).

In her early work, Ainsworth (nee Salter) used the term *secure base* to refer to the security of optimal care-giving, from which a child may explore and experience the world (Salter, 1940 as cited in Bretherton, 1992). Using ethological methods of observation she visited 28 Ugandan mother-infant dyads in their homes fortnightly over a 9-month period. Although this study was originally designed to examine infants’ responses to weaning and separation from their mothers, observation led Ainsworth to examine the process through which an attachment bond developed. In particular, she found evidence to support the premise that an infant uses his/her mother as a secure base from which to explore the world. In doing so she identified three distinct groups of attachment patterns in infants, distinguishing between those who were securely attached, insecurely attached, and not-yet-attached (Ainsworth, 1967).

In an attempt to replicate and extend these findings, Ainsworth recruited 106 pregnant mothers in Baltimore, Maryland, USA. After birth, the mother-infant dyads were visited every three weeks for the first year of life in order to determine whether maternal behaviour was associated with infant development (Bretherton, 1992). As part of this research and based on these hundreds of hours of observation, Ainsworth and Wittig (1969) developed the Strange Situation procedure.

**Measuring caregiver-infant attachment.** The Strange Situation is a reliable and valid laboratory-based assessment of patterns of caregiver-infant attachment (Ainsworth et al., 1978). The eight-episode procedure includes two separation and reunion episodes designed to heighten attachment behaviour. Within this context the trained observer may determine individual differences within the attachment
relationship with emphasis on how the relationship facilitates regulation of infant emotion and exploration (Carlson & Sroufe, 1995). Specifically, by examining organisation of infant behaviour across the domains of proximity seeking, contact maintenance, resistance, and avoidance the attachment relationship can be classified as anxious-avoidant (A), secure (B), or anxious-resistant (C). The anxious-avoidant classification is characterised by infants who avoid their mothers during the reunion episodes of the Strange Situation, and mothers who tend to be angered and irritated by their infants or rejecting of them. Secure (B) classifications are characterised by infants who exhibit positive behaviours towards their mothers and use them as a secure base from which to explore, and mothers who are sensitive to their infant’s needs. Finally, the anxious-resistant (C) groups are characterised by infants who cry often, and whose anxiety prohibits them from exploration and whose mothers are less responsive to infant signals and crying than mothers of B group infants (Ainsworth et al., 1978). Recognising that a small proportion of infants did not fit into any of these categories, lacking a coherent pattern of attachment behaviours, Main and Solomon (1990) expanded on this research and added a fourth category, disorganised/disoriented (D). Disorganised/disoriented classifications are characterised by infants whose behaviour lacks an observable goal or explanation and parents who exhibit frightened or frightening behaviour such as severe anxiety or contradictory signals (Main & Solomon, 1990).

**Caregiver-infant attachment and stress responses.** The history of these early relationships manifest in the way infants and children experience their caregiver, themselves and others they encounter. That is, a child who has experienced consistently sensitive and responsive caregiving develops an internal working model of their caregiver as someone who is trustworthy, reliable and emotionally available. Through
this experience they develop trust in their own worth and that of others they encounter. Conversely, infants who have experienced inconsistent, intrusive, or unresponsive caregiving develop less confidence in their caregiver’s ability to meet their needs, resulting in an insecure attachment style. This uncertainty becomes internalised as mistrust and negative beliefs about themselves, their caregiver and others (Belsky & Fearon, 2002). As noted earlier, in contrast to the secure and insecure attachment classifications, caregivers of infants with disorganised attachment classifications interact with their infants in a fearful or frightening way which is experienced as threatening to the infant. As such, a paradox develops whereby the source of threat to the infant or child is also the source of comfort. This paradox results in the infant developing incoherent or disorganised psychological and behavioural responses which are thought to be the earliest signs of mental health problems (Bernier & Meins, 2008; Main & Solomon, 1990).

Consistent with Attachment Theory the quality of early interactions between caregivers and infants has been described as a foundation for the development of the individual at the biological, as well as at psychological and social levels (Gunnar, 2006; Sroufe, 1996). Because associations between caregiving, infant emotional regulation and HPA function are expected to be strong, some researchers have called for inclusion of measures of physiological responses in order to validate the assessment of attachment classifications (Spangler & Grossmann, 1993).

Researchers have studied whether cortisol reactivity coincides with caregiver-infant attachment relationships. In one early and influential study, researchers examined the relationship between cortisol reactivity, temperament and attachment classification, as measured by the Strange Situation, in 66 13-month-old infants (Gunnar, Mangelsdorf, Larson & Hertsgaard, 1989). Gunnar and colleagues found that infants
who were prone to distress exhibited greater cortisol responses during the Strange Situation when compared to those who showed less distress. However, they failed to find any significant difference in cortisol reactivity when infants in different attachment categories (i.e., A, B1-B2, B3-B4, and C) were compared.

Expanding on this study other researchers measured cortisol before, during and after the Strange Situation in 41 infants (Spangler & Grossmann, 1993). Higher cortisol responses, indicating greater stress responses, were found in insecure avoidant and disorganised infants compared to infants classified as secure in attachment. However, these findings were only significant for the disorganised group when compared to the secure group. This study was replicated in a high risk sample of 35 infants. Infants who were in the disorganised attachment classification had more elevated cortisol responses to the Strange Situation compared to all other infants (Hertsgaard et al., 1995). Finally, in a study designed to examine the attachment relationship as a moderator of behavioural inhibition (a measurement of temperament) and cortisol responses to stress, Nachmias and colleagues (1996) found that children who were classified as insecurely attached to their caregiver and who rated highly on behavioural inhibition exhibited greater cortisol responses to the Strange Situation compared to (1) behaviourally inhibited children who were classified as securely attached to their caregiver and, (2) children who rated low on behavioural inhibition and were classified as securely or insecurely attached (Nachmias, Gunnar, Mangelsdorf, Parritz & Buss, 1996). Infant coping was negatively, and maternal behaviour was positively, related to infant cortisol reactivity. Nachmias et al. suggested that mothers of infants who were behaviourally inhibited and insecurely attached interfered with their infants’ attempts to cope compared to the mothers of infants who were behaviourally inhibited and securely attached and those who were rated as low on behavioural inhibition. Importantly these
findings indicate that cortisol responses during the Strange Situation may be contingent on attachment classification and infant temperament.

Findings are not completely consistent across studies, however, some discrepant findings may be explained by methodological differences. Specifically, Gunnar et al. (1989), who found no difference in cortisol reactivity between infants in different attachment classification groups, measured cortisol immediately after the Strange Situation, prior to the peak of the cortisol response. In the years since this study was conducted, new knowledge emerged showing that it takes approximately 20 minutes before the full magnitude of a cortisol response can be measured in saliva (de Weerth & Buitelaar, 2005). Further, Hertsgaard et al. (1995), who found greater cortisol increases for insecurely attached infants, failed to control for time of day of assessment and did not establish a baseline or pre-test pattern of cortisol prior to the Strange Situation. The current cortisol collection protocol is to establish a baseline level based on at least two samples prior to the day of experimentation. In order to eliminate the effects of diurnal patterns these samples are taken in the afternoon when collecting from mothers during pregnancy (de Weerth and Buitelaar, 2005) and at least 45 minutes after wakening in infants (see Larson, Gunnar & Hertsgaard, 1991). Measures of reactivity are taken approximately 20 minutes after the stressor and subsequently, recovery rates are assessed at 15-minute intervals (de Weerth & Buitelaar, 2005).

**Summary and Limitations of Previous Research addressed in the Current Study**

Cumulatively, studies of humans and non-human primates lend support to the idea that elevated psychosocial stressors, maternal mental health problems and maternal cortisol during pregnancy can exact a detrimental effect on the developing foetus and continue to have negative influences after birth. It seems that this is most likely when the stress occurs during critical periods of development. More specifically, activation of
the HPA axis and timing of the stressor are implicated in the teratogenic effect of stress. Moreover, these effects may permanently alter infant development, emotional regulatory capacity and behaviour. A recent review has suggested these changes may have evolutionary benefits such as increased risk taking and easily distracted attention in males (i.e., Conduct Disorder and Attention Deficit Hyperactivity Disorder) and increased vigilance and avoidance (i.e., anxiety) in females (Glover, 2011), each of which would be of benefit to the species under threat of attack. Glover noted that these effects were observed over a range of stress, rather than that observed in the Clinical range, indicating a need for clarification of these processes, regardless of the risks or benefits that may occur as a consequence of stress.

Although carefully controlled animal studies are suggestive of how human maternal stress could influence the developing foetus and have repercussions for infant development during the first year of life (e.g., Clarke et al., 1994; Schneider et al., 1992; Schneider et al., 1999), and similar research has been conducted with humans, the methodologies used have had some limitations. Two of the main limitations identified in the literature have been failure to attend to developmental timing across species, and the use of retrospective designs and self-report measures (Huizink, Mulder & Buitelaar, 2004). The latter may be particularly important as findings have frequently shown error in participants’ retrospective reports. These findings have included underestimation of mental health symptoms (an der Heiden & Krumm, 1991), infections during pregnancy (Voldsgaard et al., 2002) and alcohol consumption (Searles, Helzer, Rose & Badger, 2002), as well as different reports of coping (Todd, Tennen, Carney, Armeli & Affleck, 2004) when compared to reporting of events closer in time. Further, research has shown that stressful events occurring later in pregnancy are perceived to be less stressful than those occurring earlier in pregnancy (Glynn, Dunkel-Schetter, Wadhwa & Sandman,
indicating the importance of a longitudinal, prospective design that can identify and examine changes over time. Only two recent studies have addressed these limitations by commencing data collection in the first trimester of pregnancy (e.g., Rothenberger, Moehler, Reck & Resch, 2011) and at fifteen weeks gestational age (e.g., Davis et al., 2011).

More recently, researchers have aimed to address these difficulties by measuring mothers’ psychological stress and cortisol during various stages of pregnancy and examining infants after birth. Higher levels of maternal cortisol during pregnancy have been associated with lower birth weight and shorter body length (Bolten et al., 2011), immature physical and neuromuscular maturation in boys (Ellman et al., 2008), and larger amygdala volume and more affective problems in girls (Buss et al., 2012). Despite a growing body of evidence to support the premise that higher levels of maternal cortisol are involved in “programming” aspects of foetal development, to date, there is a paucity of research commencing in the first trimester of pregnancy, a period of rapid cell division.

Researchers have defined prenatal stress through three main mechanisms: 1) stressful conditions, 2) evaluation or cognitive appraisal of the stressful conditions, and 3) stress responses such as anxiety and depression (Hammen, 2005; Saunders, Lobel, Veloso & Meyer, 2006). An early review highlighted the need to expand on previous definitions (de Weerth & Buitelaar, 2005). The use of different definitions and assessment tools to measure prenatal stress between studies indicate the need to include a physiological measure of stress to validate self-report data, provide a level of standardisation, and clarify the role that maternal stress responses play in deleterious infant outcomes. Further, findings by Hertsgaard et al. (1995) indicate that coping may play an important role in attenuating stress responses. These suggestions highlight the
need for a multidimensional definition of prenatal maternal stress that recognises the importance of physiological responses, developmental timing and maternal coping efficacy. With this in mind, prenatal maternal stress may best be operationalised through this multidimensional definition: 1) the occurrence of stressful events, 2) self-appraisal of the distress caused by each event, 3) perceived capacity to cope with stressful events (i.e., coping efficacy), 4) mental health outcomes linked with stress, including depressive symptoms and anxiety, and 5) cortisol (a physiological indicator of psychological stress).

There is accumulating evidence from primate and human studies that infant stress responses, as measured by cortisol (basal level, reaction, and regulation), in the first year of life are an outcome of foetal glucocorticoid exposure and mothers’ experiences of stressful events, coping and mental health during or shortly after pregnancy. Further, evidence from human studies indicates that optimal parenting during the first months of life can moderate stress responses in infants (Hertsgaard et al., 1995) and promote a secure caregiver-infant attachment relationship (Gunnar, 2006). Although there are limited prospective studies of prenatal stress and infant cortisol reactivity and behaviour in humans commencing in the first trimester, findings from recent studies are beginning to show that some associations found in primates are likely to occur among humans. Yet, the timing of prenatal stress in relation to differences in infant stress responses and basal levels of cortisol have not yet been clearly defined. Huizink and colleagues (2003) suggested that exposure to stress in early gestation had the most profound effects on development in rhesus monkeys. In humans the foetal HPA axis does not develop until the second trimester of pregnancy (Nader, 2004), leaving the developing foetus particularly vulnerable to exposure of maternal cortisol during the first trimester of pregnancy when it is most vulnerable to teratogens.
Further, it has been suggested that stress is appraised differentially by mothers throughout pregnancy (Glynn et al., 2004). However, little is known about how this may affect cortisol levels or the developing foetus. Researchers have called for further investigation into the developmental sequelae of anomalous HPA responsivity in infants. Keenan, Gunthorpe & Grace (2007) suggested “What needs to be determined is the point in development during which alterations or atypicalities in the stress response system are observed” (p. 135). To date, there has been only one published prospective study examining psychosocial stress of pregnant women (assessed via cortisol and self-report) commencing in trimester one and continuing across all trimesters of pregnancy (e.g., Rothenberger et al., 2011) and one study commencing data collection at 15 weeks gestation and continuing throughout pregnancy (Davis et al., 2011). One aim of the current study was to examine the effects of developmental timing of stress on basal levels of infant cortisol, and infant cortisol reactivity to, and recovery from, stressors within the first year of life. Further, it was expected that an infant’s stress reactivity and regulation would be associated with attachment classification at one year of age, but that this association may be complicated by maternal mental health in the first months of life.

**The Current Study of First Time Mothers**

The primary goal of this longitudinal study was to test a series of hypothesised concurrent and prospective associations between prenatal and postnatal maternal stress, prenatal maternal cortisol levels, infant cortisol reactivity, and regulation, during the first year of life, and caregiver-infant attachment at 12 months of age. Maternal depressive, anxiety and stress symptoms, pregnancy-related anxiety and coping efficacy were considered. The study was organised into four parts.
In Study 1A, the general purpose was to address methodological limitations of previous research relating to the developmental timing of stress and retrospective reporting. This involved testing associations between measures completed by first time mothers in their first, second and third trimesters of pregnancy. More specifically, mothers’ experiences of stress, depression, and anxiety symptoms, pregnancy-related anxiety, coping efficacy, and cortisol during pregnancy were collected to address three main aims. First, to examine whether maternal reports of stress and coping measured early in pregnancy were associated with subsequent cortisol samples. Second, whether these same variables were associated with stress, depression, and anxiety symptoms and pregnancy-related anxiety during pregnancy and third, to examine patterns of stress and coping early in pregnancy to determine associations with cortisol and mothers' mental health later in pregnancy.

In Study 1B, the focus was on accounting for mothers' postnatal depressive symptoms 2-months after birth by focusing on prenatal factors. These prenatal factors were maternal cortisol, daily stress, anxiety, stress, depressive symptoms, and pregnancy-related anxiety measured in each trimester of pregnancy. Linkages between variables over time were determined using structural equation modelling (SEM), and pathways were identified through growth curve modelling.

In order to test the foetal programming hypothesis, Study 1C involved testing associations between measures completed by pregnant women prior to birth and infant cortisol measured at 2, and 4 months after birth.

In the final study, Study 1D, maternal mental health, the development of infant stress regulation and emergent coping and caregiver-infant attachment measured 12 months after birth were examined. In this study, associations of attachment with prenatal maternal measures and infant cortisol were examined.
CHAPTER 3

Study 1A: Coping Moderates the Associations of Daily Stress with Cortisol and Anxiety during Pregnancy

Research in the area of maternal mental health and well-being during pregnancy has increased over the past 15 years and, recently, has focused on maternal mental health during pregnancy because of its possible predictive utility for identifying women at risk of emotional and social problems after birth. Much of this research has emerged because of concerns over the high rates of, and significant disability associated with, postnatal depression and the need to better identify women at the greatest risk as early as possible. Meta-analyses have suggested postnatal depression occurs in approximately 13 percent of women (O’Hara & Swain, 1996; Beck, 1996) and studies of depression during pregnancy suggest the rates of mood disorder during the later stages of pregnancy are almost double those of women in the general population (Gaynes et al., 2005). Despite depression being acknowledged as the most common health problem in women (Kessler et al., 2005) it is still thought to be under-diagnosed and under-treated (Holden, 1996; Vesga-Lopez et al., 2008). Subclinical levels of depressive symptoms can also cause significant distress and estimates suggest that up to 50 percent of women experience a degree of depressive symptoms during pregnancy (Gaynes et al., 2005).

Some of the most commonly reported psychosocial risk factors for depression include poor social support, stressful life events, poor partner relationship, and history of major depressive disorder and/or anxiety (Garcia-Esteve et al., 2008; Milgrom et al., 2008; O’Hara & Swain, 1996). Risk factors with biological foundation are also of importance. These have included a history of premenstrual disorder (Garcia-Esteve et al., 2008), and irritable mood prior to menstruation (Limlomwongse & Liabsuetrakul,
indicating that endocrine factors may also play an important role in the onset of mental health problems prior to birth.

**Maternal Stress and Mental Health during Pregnancy**

Recently, researchers have suggested prenatal depression may have a similar or greater impact on mother-infant well-being when compared to the impact of postnatal depression (Bonari et al., 2004; Koleva, Stuart, O’Hara & Bowman-Reif, 2011). Prenatal depression has been linked to poorer obstetric care, higher rates of pre-eclampsia, slower foetal growth, increased use of pain relief during labour, preterm delivery, low birth weight, and higher cortisol levels and disorganised sleep patterns in newborns (Chung, Lau, Yip, Chiu & Lee., 2001; Diego et al., 2009; Diego et al., 2004; Kurki et al., 2000; Steer, Scholl, Hediger & Fischer, 1992). There is consensus that prenatal depression is the biggest predictor of postnatal depression, however our understanding of the mechanisms linking prenatal mental health, more broadly, to poorer postnatal outcomes is still debated. Prenatal stress and anxiety have also been linked to poorer outcomes for mothers and infants. Prenatal anxiety has been shown to predict postnatal depression independently of prenatal depression, as well as being associated with behavioural and emotional problems in children (O’Connor, Heron, Golding, Beveridge & Glover, 2002), whereas prenatal stress has also been found to partially account for many prenatal and early life experiences including pre-term birth, low birth weight (Killingsworth Rini, Wadhwa, Dunkel-Schetter & Sandman, 1999; de Weerth, van Hees & Buitelaar, 2003) and, slower rates of behavioural recovery from stressors (Davis, Glynn, Waffam & Sandman, 2011).

Researchers have suggested a complicated bidirectional unfolding of depression and anxiety during pregnancy whereby depression early in pregnancy predicted anxiety later in pregnancy, which in turn predicted postnatal depression (Skouteris, Wertheim,
Rallis, Milgrom & Paxton, 2009). Other researchers suggested prenatal depression may be stress related, particularly when stress is high during the first trimester of pregnancy (Da Costa, Larouche, Drista & Brender, 2000). Expanding this stress-symptomatology model even further, researchers acknowledge endocrine factors may best account for the relationship between prenatal stress, anxiety and depression and this, may in turn, explain poorer maternal and infant outcomes (Wadhwa, 2005; Wadhwa, Dunkel-Schetter, Chicz-DeMet, Porto & Sandman, 1996).

**Pregnancy-Related Anxiety**

Although previous studies have focused on associations between general anxiety and depressive symptoms, pregnancy and childbirth offers an experience with unique concerns and fears that require complex physical, psychological and social adjustment for many women. As such, some researchers have suggested general measures of depression and anxiety do not wholly capture the psychological challenges experienced by women during and after pregnancy. Developing this idea, Huizink and colleagues argued that pregnancy-related anxiety is a distinct form of anxiety (Huizink, Mulder, Robles de Medina, Visser & Buitelaar, 2004). To test this hypothesis, 230 nulliparous pregnant women completed measures of pregnancy-related anxiety, general anxiety and depression at three stages during their pregnancy. Pregnancy-related anxiety included three factors; fear of giving birth, fear of bearing a physically or mentally handicapped child and, concern about appearance. Although general measures of anxiety (i.e., State-Trait Anxiety Inventory) and depression (i.e., Edinburgh Postnatal Depression Scale) accounted for between 8 and 27% of the variance of pregnancy-related anxiety in early to mid pregnancy, this relationship was not found later in pregnancy. Moreover, mothers with higher pregnancy-related anxiety scores during pregnancy had infants with more behavioural and attentional problems and developmental delays in the first
twelve months of life. Huizink concluded that pregnancy-related anxiety is a unique aspect of psychological well-being during pregnancy, which seems important as a correlate of postnatal maternal mental health and infant outcomes. More recently, pregnancy-related anxiety, measured mid-gestation, has been associated with reduced gray matter volume in middle childhood (Buss, Davis, Muftuler, Head & Sandman, 2010) indicating structural changes to brain development occur. The authors reported this may increase vulnerability to neurodevelopmental disorders and decreased cognitive function. Importantly, these findings, in healthy children, were significant after controlling for postnatal factors such as socioeconomic status and ongoing maternal stress.

**Stress and Coping**

*Cortisol: A physiological indicator of psychological stress.* One method of capturing maternal stress levels is through the hormone cortisol. Cortisol is the hormonal end-product of the Hypothalamic Pituitary Adrenocortical (HPA) axis and it is widely used as a marker of stress in research (e.g., Gunnar & Vazquez, 2006). Cortisol is present in the body in low levels during non-stress periods and at higher levels in response to stressors. However, during pregnancy a complex interplay occurs between mother, placenta and foetus resulting in cortisol levels rising as pregnancy progresses.

Throughout pregnancy maternal cortisol passes through the placenta into foetal circulation. Much of the maternal cortisol is metabolised by the enzyme 11β-Hydroxysteroid dehydrogenase-2 which is heavily expressed in the placenta. However, approximately 40% of maternal cortisol (Gitau, Cameron, Fisk & Glover, 1998; Gitau, Fisk, Teixeira, Cameron & Glover, 2001) passes into foetal circulation where it has the ability to influence the developing foetus prior to development of the infant HPA axis.
(Nader 2004). Around the second trimester of pregnancy, as the infant HPA axis matures, transportation of maternal cortisol reduces and the infant cortisol secretion increases (Keller-Wood and Wood, 2001). During the third trimester of pregnancy foetal cortisol levels control parturition and it has been suggested that pre-term birth is a sign of dysregulation of the parturition process caused by these endocrine responses (Glynn, Dunkel-Schetter, Hobel & Sandman, 2008).

Whereas stress theorists have typically defined stress as a pressure that exceeds perceived coping resources (Lazarus & Folkman, 1984), more recently it has been defined as any event that increases the secretion of glucocorticoids (Heuser & Lammers, 2003). Research has shown that depressed mood is characterised by increased HPA axis activity which accounts for the elevated cortisol levels found in depressed people (Van den Berg, Van Calster, Smits, Van Huffel & Lagae, 2007). Researchers have suggested that cortisol is a credible mechanism through which maternal mental health during pregnancy impacts foetal development (Giesbrecht, Campbell, Letourneau, Kooistra & Kaplan, 2012). However not all research has supported this hypothesis (Goedhart, Vrijkotte, Roseboom, Van der Wal & Cuijpers, 2010).

**Coping with stress during pregnancy.** Most prominent stress and coping theories recognise that while stress can impact on psychological well-being, individual coping responses play an important role in moderating stress responses and facilitating adjustment. Coping has been described as “constantly changing cognitive and behavioural efforts to manage specific external and/or internal demands that are appraised as taxing or exceeding the resources of the person” (Lazarus & Folkman, 1984, p. 141). Few studies have examined the role of coping on physiological stress and maternal well-being during pregnancy. Urizar et al. (2004) found that simple instructions to reduce stress lowered symptoms of depression and levels of morning
cortisol in pregnant women. However, this study did not take into account changes in cortisol throughout pregnancy and examined women only once during pregnancy with participants at various stages of gestation. Additionally, this research was conducted over a brief period of nine days. Da Costa and colleagues (2000) found that depressed women in the pre- and post-birth period used more emotion-focused coping than non-depressed women. However, studying women in the postpartum period other researchers have found that emotion-focused coping was a useful mechanism for regulating emotional distress and, further, lower levels of this style of coping actually predicted suicidal ideation (Doucet & Letourneau, 2009). Yali and Lobel (2002) reported that coping strategies for distress reduced over time and showed little effect in moderating distress over a ten-week period during pregnancy.

This body of research highlights the need for a multidimensional definition of prenatal maternal stress that recognises the importance of physiological responses, maternal mental health and coping efficacy. With this in mind, prenatal maternal stress may best be operationalised through this multidimensional definition: 1) the occurrence of stressful events, 2) self-appraisal of the distress caused by each event, 3) perceived capacity to cope with stressful events (i.e., coping efficacy), 4) mental health outcomes linked with stress, including depressive symptoms and anxiety, and 5) physiological stress responses.

Taken together, it is important to further investigate links between maternal stress, coping, stress physiology, and symptoms of mental health problems during pregnancy by focusing on each trimester and examining patterns across the entire prenatal period. This was the general intent of the current study.

Timing of Stress and Mental Health during Pregnancy
In humans, research has shown that stress (Da Costa et al., 2000) and depression (Da Costa et al., 2000; Skouteris et al., 2009) early in pregnancy are each linked to poorer maternal mental health later in pregnancy and in the postpartum. Further, stressful events occurring later in pregnancy are perceived to be less stressful than those occurring earlier in pregnancy (Glynn, Dunkel-Schetter, Wadhwa & Sandman, 2004) indicating the importance of a longitudinal, prospective design that can identify and examine changes over time. Previous research in this area has been criticised for retrospective design, the use of self-report measures, and failure to examine all trimesters of pregnancy.

**Study Aims and Hypotheses**

While addressing previous methodological limitations relating to the developmental timing and self-reporting of stress, there were three primary aims of the current study. The first aim was to examine how self-reported stress and coping efficacy early in pregnancy were associated with cortisol later in pregnancy. The second aim was to determine whether self-reported stress and coping efficacy were related to depression, anxiety, and pregnancy-related anxiety in each pregnancy trimester. The third aim was to determine whether patterns of stress and coping early in pregnancy predicted cortisol levels and whether cortisol in turn predicted poorer mental health outcomes later in pregnancy. Together, these aims test the importance of perception of daily stress (hereafter “daily stress”) and coping on physiological stress responses and mothers' mental health during pregnancy.

The specific hypotheses of this study were:

1. Mothers’ daily stress will be positively correlated with cortisol levels.

2. Daily stress will be positively associated with mothers’ mental health symptoms (depression, anxiety, and pregnancy-related anxiety).
3. Coping efficacy will moderate associations between frequency of daily stressors, daily stress and cortisol, with a weaker association between daily stress and cortisol expected under conditions of more adaptive coping.

4. Coping efficacy will moderate associations between daily stress and stress symptoms, with a weaker association between daily stress and stress symptoms expected under conditions of more adaptive coping.

5. Stress and coping in trimester one will be related to cortisol in trimester two which, in turn, will predict poorer mental health outcomes later in pregnancy.
Method

Participants and Procedure

The following study took place between August, 2008 and December, 2011. Following approval by the University Human Ethics Committee of Griffith University this research was conducted in accordance with the National Statement on Ethical Conduct in Research Involving Humans. Prior to inclusion in the study, participants were given written and verbal information outlining the requirements of the study and signed consent forms prior to data collection. Participants were informed that they could withdraw from the study at any time.

Forty-five nulliparous pregnant women were recruited through newspaper advertisements and general medical practitioner’s offices. Following an initial screening process, women were eligible to participate in the study if they were between 7 and 13 weeks gestation with no other biological children and not suffering from skin or inflammatory disorders requiring medical treatment, or diseases known to affect production of cortisol (e.g., Addison’s Disease, Cushing’s Syndrome). Three women were excluded when the gestational age of the foetus was reassessed later in pregnancy indicating that they were not in the first trimester of their pregnancy at commencement of the study and two women experienced miscarriage. The final participant group comprised 40 women aged between 21 and 42 years, with a mean age of 30.6 years (SD = 5.1 years). The average length of gestation on entry to the study was 9.8 weeks (SD = 2.0). After the initial meeting (T1) data were collected between 21-23 weeks gestation (T2; 36 women collected at 21 weeks, three participants collection was postponed by one week due to headcolds or flu symptoms and one was postponed by 2 weeks due to vacation), and at 32 weeks gestation (T3). One participant gave birth prior to completing T3 data. The following measures were administered at each time point.
Measures

**Maternal cortisol.** Maternal saliva samples were collected in the afternoon between 4 and 5 pm on 2 consecutive days. Sampling was taken in the afternoon to minimise the effects of circadian variations (de Weerth & Buitelaar, 2005). Participants were asked to refrain from eating or drinking for at least 20 minutes prior to producing a saliva sample into a vial. When possible, and in every case during trimester 2 and 3, participants were sent a text message at approximately 4pm reminding them to produce a sample on the days scheduled for saliva collection. Most participants responded by text on completion of sample collection. Participants were asked to report if they were unwell or using medication at each stage of data collection. Samples from three participants were postponed by one week due to head colds or flu symptoms. All participants were asked to postpone collection for 24 hours if they had slept during the afternoon on the day collection was due. Samples were frozen at home until collected by the researcher. Samples were transported on ice and frozen at -20°C until analyses were performed. All samples from the same research participant were run, in duplicate, in the same assay using Salimetrics salivary cortisol enzyme immunoassay kit according to manufacturer’s protocol.

**Daily Stress.** The 58-item Daily Stress Inventory (Brantley, Waggoner, Jones & Rappaport, 1987) is a self-report measure designed to measure life events during the 24 hours prior to administration of the questionnaire, and rated the respondent’s subjective stress of these events. Participants were asked to indicate whether a variety of events had occurred. Sample questions included “Had your sleep disturbed” and “Argued with partner”. Responses to each item ranged from 1 (occurred but was not stressful) to 7 (caused me to panic), response “X” was endorsed if the item did not occur. Three scores were calculated for each participant: 1) frequency (FREQ) scores were calculated by
summing the frequency of life events reported; 2) sum (SUM) scores were calculated by summing the individual’s rating of subjective distress; and 3) Average Impact Rating (AIR) of the events was calculated by dividing the SUM by the FREQ. The DSI has been shown to have convergent validity with samples of urinary cortisol in a study of 18 males indicating that it is a reliable and valid measure of daily stress (Brantley, Dietz, McKnight, Jones & Tulley, 1988). Cronbach’s $\alpha$’s ranged from .92 to .95 for frequency scores and .91 to .96 for SUM scores throughout pregnancy. Only FREQ and AIR were used in the current study.

**Coping efficacy.** Maternal coping self-efficacy was measured using the Coping Self-efficacy (CSE) scale (Chesney, Neilsands, Chambers, Taylor & Folkman, 2006). This 13-item, factor-analytically derived measure consisted of three subscales: Participants were asked the stem question “When things aren’t going well for you, or when you’re having problems, how confident or certain are you that you can do the following:” use problem-focused coping (six items; e.g., “Break an upsetting problem down into smaller parts”), stop unpleasant emotions and thoughts (four items; e.g., “Make unpleasant thoughts go away”), and get support from friends and family (three items; e.g., “Get friends to help you with the things you need”). Responses ranged from 0 (cannot do at all) to 10 (certain I can do). Responses were summed for a total CSE score with higher scores indicating higher coping self efficacy. Cronbach's $\alpha$'s ranged from .82 to .89 for use problem-focused coping, .94 to .95 for stop unpleasant emotions and thoughts, and .69 to .85 for get support from friends and family throughout pregnancy.

**Depression, anxiety and stress.** Maternal depressive, anxiety and stress symptoms were measured using the 21-item Depression Anxiety Stress Scales (DASS; Lovibond & Lovibond, 1995). This brief measure was chosen 1) due to its ability to
discriminate between three negative mood states and 2) due to the availability of Australian norms. The depression subscale consisted of seven items designed to assess negative emotional state (e.g., self-depreciation, anhedonia and lack of interest). The anxiety subscale, designed to measure situational and subjective anxiety including autonomic arousal and muscular effects, consisted of seven items. The remaining seven items formed the stress subscale. These were designed to assess chronic, non-specific arousal (e.g., difficulty relating and getting upset easily). Participants indicated how much each statement applied to them during the prior week. Responses ranged from 0 (did not apply to me at all) to 3 (applied to me very much). Scores were totalled and doubled. Higher numbers indicated higher levels of negative affect, anxiety and stress. The DASS is a widely used measure available in the public domain and has previously been used in studies of pregnant women (e.g., Reid, Power & Cheshire, 2009). Cronbach’s \( \alpha \)'s ranging from .70 to .80 for depression, .53 to .72 for anxiety, and .77 to .85 for stress during pregnancy.

**Pregnancy-related anxiety.** Pregnancy-related anxiety was measured using the short form of the Pregnancy-related Anxiety Questionnaire-Revised (PRAQ-R; Huizink, 2000). This 10-item, self-report measure, consisted of three factor-analytically derived subscales designed to measure: Fear of giving birth (3 items; e.g., “I am worried about not being able to control myself during labour and fear that I will scream”), fear of bearing a physically or mentally handicapped child (4 items; e.g., “I am afraid that our baby will suffer from a physical defect or worry that something will be physically wrong with the baby”), and concern about one’s appearance (3 items; e.g., “I am worried about the fact that I shall not regain my figure after delivery”). Response options for each item ranged from 1 (absolutely not relevant) to 5 (very relevant). Items on each subscale were summed with higher scores indicating higher levels of
pregnancy-related anxiety. Across T1, T2 and T3, Cronbach's \( \alpha \)'s of .82 to .84 were found for fear of giving birth, .86 to .91 for fear of bearing a physically or mentally handicapped child, and .87 to .90 for concern about one’s appearance.
Results

Data Analyses

Analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 19. There were four sets of analyses. In the first set, repeated measures ANOVA was used to examine the mean levels and patterns of daily stress, cortisol, coping and mental health across the three trimesters of pregnancy. In the second set of analyses, Pearson correlations between measures were calculated to examine the interrelations of stress, cortisol, coping and mental health in each trimester. In the final two sets of analyses, multiple regression was used to examine 1) associations of stress and coping with cortisol and 2) associations of stress and coping with women's mental health. These regression models were also used to test whether women's greater use of adaptive coping strategies moderated (i.e., buffered) the impact of stress on either cortisol or mental health. Interaction effects (e.g., daily stress × coping strategy) were used and variables were centred prior to analysis. Hence, moderator (i.e., interaction) effects were formed by multiplying centred values of stress and coping. For each significant interaction, simple slopes analysis was conducted using the unstandardised coefficients to further examine it. High and low scores of daily stress and coping were calculated as one standard deviation above and below the mean, respectively.

Descriptive Statistics of Participants and Measures

Participants were predominantly middle class of Australian and European heritage. Participants were predominantly married (65%) or in defacto relationships (30%) with an annual household income greater than or equal to Aus $ 75 000 (68%; sample range 6-25K per annum (2 people) - >100K per annum). Ninety percent of mothers and 76% of fathers had completed high school and 75% of mothers had pursued tertiary education (e.g., trade certificate, diploma, undergraduate or
postgraduate degree). At commencement of data collection, only one participant did not work outside of the home.

First trimester data (T1) indicated five participants had consumed alcohol (range 1 - 4 standard drinks per week) and one smoked cigarettes (5 per week). Thirty-one women reported experiencing morning sickness (range less than once per week to more than ten times per day). One participant was diagnosed with hyperemesis gravidarum and received medical treatment to reduce nausea and prevent dehydration throughout pregnancy. At T2, ten participants reported alcohol consumption (range 1 – 4 standard drinks per week), the participant who smoked increased consumption to 25 cigarettes per week and 16 participants reported experiencing morning sickness. By T3, nine participants reported alcohol consumption (range 1 – 3 standard drinks per week), the participant who smoked continued to smoke 25 cigarettes per week and nine participants reported experiencing morning sickness. No participants reported use of marajuana or other illicit substances at any stage during pregnancy.

Diet, exercise and sleep were not systematically examined in the current study. Also, no further health information was collected systematically, however at each stage of data collection participants were asked about their health and no significant illnesses were recorded. Descriptive statistics for measures used throughout pregnancy are shown in Table 1.
Table 1

*Descriptive Statistics of Measures During Pregnancy (N = 40 at T1 and T2, N = 39 at T3)*

<table>
<thead>
<tr>
<th>Measure</th>
<th>Time</th>
<th>M</th>
<th>SD</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
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<td>Cortisol (µg/L)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td>.73</td>
<td>.39</td>
<td>.26</td>
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<tr>
<td></td>
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<td>1.65</td>
<td>.31</td>
<td>9.18</td>
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<tr>
<td></td>
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<td>.97</td>
<td>.23</td>
<td>4.09</td>
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<tr>
<td></td>
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</tr>
<tr>
<td></td>
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<td>11.21</td>
<td>6.00</td>
<td>58.00</td>
</tr>
<tr>
<td></td>
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<td>21.18</td>
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<td>60.00</td>
</tr>
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<td></td>
<td></td>
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<td></td>
</tr>
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<td>.76</td>
<td>1.07</td>
<td>4.46</td>
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<td>.77</td>
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<tr>
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<tr>
<td></td>
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<tr>
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<td>3.91</td>
<td>0.00</td>
<td>16.00</td>
</tr>
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<td>DASS Anx</td>
<td></td>
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<tr>
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<tr>
<td></td>
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<td>0.00</td>
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<tr>
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<td>43.05</td>
<td>7.93</td>
<td>21.00</td>
<td>58.00</td>
</tr>
<tr>
<td></td>
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<td>8.32</td>
<td>26.00</td>
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</tr>
<tr>
<td></td>
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<td>43.13</td>
<td>8.40</td>
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<td>60.00</td>
</tr>
<tr>
<td>Coping (Get Support)</td>
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<td></td>
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<tr>
<td></td>
<td>T1</td>
<td>22.98</td>
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</tr>
<tr>
<td></td>
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<td></td>
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<td>5.07</td>
<td>13.00</td>
<td>30.00</td>
</tr>
<tr>
<td>Coping (Stop Thoughts)</td>
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<td></td>
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<tr>
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</tr>
<tr>
<td></td>
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<td>25.59</td>
<td>8.48</td>
<td>4.00</td>
<td>40.00</td>
</tr>
<tr>
<td>PRA (Birth)</td>
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<tr>
<td></td>
<td>T1</td>
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<td>3.16</td>
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<td>7.28</td>
<td>2.85</td>
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<td>14.00</td>
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<tr>
<td>PRA (Child Health)</td>
<td></td>
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<td>PRA (Appearance)</td>
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</table>
Daily Stress, Coping, and Symptoms of Mental Health Problems during Pregnancy

To examine the patterns of daily stress, coping, cortisol, general anxiety, pregnancy-related anxiety and depressive symptoms over time (i.e., over three trimesters), a series of one-way repeated measure ANOVAs were performed. A main effect of time was found for cortisol, $F(1, 37) = 125.82 \, \mu g/L, p < .01$. Inspection of 95% confidence intervals revealed that participants had lower levels of cortisol during their first trimester, $T1 \ (M = 0.74 \, \mu g/L, 95\% \ CI = 0.61 - 0.86)$ than they did at $T2 \ (M = 1.55 \, \mu g/L, 95\% \ CI = 1.00 – 2.11)$ or $T3 \ (M = 1.71 \, \mu g/L, 95\% \ CI = 1.39 – 2.02)$. There was no difference between mean cortisol levels at $T2$ and $T3$ of pregnancy.

There was no within subject change in the mean level of depression, general anxiety, stress or coping efficacy across the three trimesters of pregnancy. However when pregnancy-related anxiety was examined a significant main effect for time was noted for fear of giving birth, $F (2, 39) = 4.30, p < .05$. Inspection of 95% confidence intervals revealed women reported significantly more fear of giving birth in $T3$ of pregnancy ($M = 7.28, 95\% \ CI = 6.36 - 8.21$) compared to $T1$ and $T2$ ($M = 6.38, 95\% \ CI = 5.35 - 7.42; T2 \ M = 6.38, 95\% \ CI = 5.45 - 7.32$, respectively). A main effect of time approached significance for fear for child health, $F (2, 39) = 2.65, p = .08$. This indicated a trend towards women reporting more fear for their child’s health at $T1$ ($M = 9.00, 95\% \ CI = 7.87 – 10.13$) than they did at $T2 \ (M = 8.36, 95\% \ CI = 7.12 - 9.60)$ or $T3 \ (M = 7.77, 95\% \ CI = 6.63 – 8.91)$. A paired sample t-test indicated women reported more fear for their child's health in $T1$ than in $T3$, $t (38) = 2.19, p < .05$.

**Simple Correlations between Measures**

Pearson's correlations between mothers’ cortisol levels and self-report measures of stress, coping efficacy, mental health symptoms and pregnancy-related anxiety in each trimester of pregnancy are shown in Table 2, Table 3, and Table 4. In support of
Hypothesis 1, mothers who reported more stress had higher levels of cortisol, but this was only significant in T2. There were no significant bivariate associations between coping efficacy and cortisol in any trimester. Cortisol was not associated with mental health symptoms in T1, but mothers with higher levels of cortisol reported more anxiety in T2 and T3 and reported more T2 pregnancy-related anxiety (fear of giving birth and fears about appearance).
Table 2

Correlations between Trimester 1 Variables (N = 40)

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*p < .05. **p < .01.
Table 3

*Correlations between Trimester 2 Variables (N = 40)*

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*p < .05. **p < .01.
Table 4

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*p < .05. **p < .01.
In support of Hypothesis 2, associations of stress with mental health symptoms were found and these were most strong and consistent in T2. Stress, depression and anxiety symptoms on the DASS were generally positively intercorrelated and DASS stress symptoms and/or daily stress were associated with higher levels of at least one subscale of pregnancy-related anxiety in each trimester.

**Coping as a Moderator of the Association between Self-reported Stress and Cortisol**

Multiple regression was used to determine whether coping moderated the relationship between cortisol levels and mothers’ stressful life events in each trimester of pregnancy (Hypothesis 3, see Table 5). Independent variables were centred prior to analysis. Moderator (i.e., interaction) effects were formed by multiplying centred values of stress and coping. Interactions between mothers’ daily stress and coping style (i.e., problem focused coping, ability to stop unpleasant thoughts and emotions, and getting support from family and friends) were tested in separate regression models for each trimester of pregnancy with cortisol level as the dependant variable.

Overall, one significant interaction effect was found when examining measures completed in T2 and three were found when examining measures in T3. At T2, women's reports of seeking support coping moderated the relationship between their stress and cortisol levels. Simple slopes analysis revealed that women who reported the highest stress and the lowest coping self-efficacy had the highest level of cortisol (see Figure 1).

In support of Hypothesis 3, all T3 styles of coping moderated the relationship between women's daily stress and cortisol level. Consistent with the expected role of coping and stress on physiological stress responses, mothers who reported high perceived impact of daily stress but also reported highest ability to cope either by
problem focussed coping, stopping unpleasant thoughts and emotions or seeking support, had the lowest T3 cortisol levels (see Figure 2, Figure 3, and Figure 4).

**Coping as a Moderator of the Associations between Daily Stress and Mental Health**

Multiple regression was used to determine whether coping moderated the relationship between daily stress and maternal mental health during pregnancy. As recommended, independent variables were centred prior to analysis (Jaccard, Wan & Turrisi, 1990). Moderator (i.e., interaction) effects were formed by multiplying centred values of stress and coping. Interactions between frequency of daily stress and coping style (i.e., problem focussed coping, ability to stop unpleasant thoughts and emotions, and getting support from family and friends) were tested in separate regression models for depression, anxiety and stress (as measured by the DASS), for each trimester of pregnancy (see Table 6). Three significant interaction effects were found in T3, but no interactions were significant in T1 or T2. The interactions indicated that each style of coping moderated the association between frequency of daily stress and anxiety.

Simple slopes analyses were conducted using the unstandardised coefficients to further examine the interaction effects. High and low scores of daily stress and coping were calculated as one standard deviation above and below the mean, respectively (see Figure 5, Figure 6, and Figure 7). Figure 5 indicates that when the frequency of daily stress was low, problem focussed coping made little difference to mothers’ symptoms of anxiety. However, when frequency of daily stress was high, women who reported higher levels of problem focussed coping reported fewer symptoms of anxiety than those who reported lower levels of coping. Figure 6 and Figure 7 indicate similar patterns for mothers who coped by stopping unpleasant thoughts and emotions or who felt confident they could seek support from family or friends.
Table 5

Results of Regressing Cortisol on Stress, Coping, and the Moderating Affect of Coping in Trimesters 1, 2 and 3 (N = 40)

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<th>Modifying Variable (MV)</th>
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*Note. The dependent variable in each model was cortisol within the same trimester of pregnancy.

*p < .05. **p < .01
Trimester 2 Frequency of Stressors Reported

Figure 1. Interaction effect of support coping and daily stress on anxiety at T2

Trimester 3 Impact of Stress

Figure 2. Interaction effect of problem-focused coping and daily stress on cortisol at T3
Figure 3. Interaction effect of stopping unpleasant thoughts and daily stress on cortisol at T3

Figure 4. Interaction effect of support coping and daily stress on cortisol at T3
Figure 5. Interaction effect of frequency of daily stressor and problem-focused coping on anxiety at T3

Figure 6. Interaction effect of frequency of daily stressors and stopping unpleasant thoughts/emotions on anxiety at T3
Figure 7. Interaction effect of frequency of daily stressors and support coping on anxiety at T3

Relationships Between Variables across the Trimesters of Pregnancy

**T1 to T2.** Multiple regression was used to determine whether T1 daily stress and coping were associated with T2 cortisol. In four regression models, there were no significant associations of daily stress (p = .13), problem focussed coping (p = .53), or stopping unpleasant thoughts coping (p = .71) with cortisol during the second trimester of pregnancy. The relationship between seeking support to cope during the first trimester of pregnancy and cortisol during the second trimester of pregnancy was also not significant, but approached it (p = .07).

To determine whether T1 cortisol was associated with symptoms of mental health at T2, six regression models were tested (i.e., one each for the DASS and PRAQ subscales of depression, anxiety, stress, fear for child health, birth and appearance). Cortisol during the first trimester was not related to depression (p = .83), anxiety (p =
.54), or stress ($p = .94$) during the second trimester. Nor was it related to mothers’ fear for child health ($p = .68$), giving birth ($p = .46$), or appearance ($p = .08$). Hypothesis 5 was not supported.

Multiple regression was used to determine whether daily stress and coping at T2 predicted cortisol at T3. In four regression models, neither daily stress ($p = .62$), problem focused coping ($p = .58$), stopping unpleasant thoughts ($p = .90$), nor seeking support to cope ($p = .82$) at T2 were related to cortisol at T3.

To determine whether T2 cortisol was associated with T3 symptoms of mental health problems (as measured by the DASS and PRAQ), six regression models were tested (i.e., one each for depression, anxiety, stress, and fear for child health, birth and appearance). In these analyses, significant relationships were found between T2 cortisol and T3 anxiety (as measured by the DASS, $\beta = .50$, $R^2 = .25$, $p < .01$), and between T2 cortisol and T3 stress ($\beta = .36$, $R^2 = .13$, $p < .05$).
### Table 6

**Results of Regressing Measures of Mental Health on Frequency of Daily Stress, Coping, and the Moderating Effect of Coping in Trimesters 1, 2 and 3 (N = 42)**

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<td>.39*</td>
<td>-.15</td>
</tr>
</tbody>
</table>

*Note.* The independent variable (IV) in every model was frequency of daily stress in the same trimester of pregnancy. Only standardised regression coefficients are reported to reduce table length, but unstandardised coefficients were used for interpreting interactions.

*p < .05. **p < .01.*
Discussion

In this study, concurrent and prospective associations of basal levels of cortisol, coping, anxiety, stress, and depressive symptoms were examined over the course of pregnancy. When data collected in Trimester 2 or 3 were analysed, the findings were consistent with the hypothesised role of coping and stress on physiological stress responses. During the second trimester of pregnancy, seeking support to cope moderated the relationship between mothers’ perceptions of the impact of their daily stress and their cortisol levels. Mothers with the highest levels of cortisol reported the most daily stress and lowest coping self-efficacy. During the third trimester of pregnancy mothers who reported high daily stress but also reported the highest coping self-efficacy, either by problem-focussed coping, stopping unpleasant thoughts and emotions, or seeking support, had the lowest cortisol levels. Additionally, all styles of coping attenuated the relationship between maternal reported frequency of daily stress and reported symptoms of anxiety during the third trimester of pregnancy. However, when frequency of daily stressors was low, coping had a non-significant association with anxiety levels. These findings support previous research (e.g., Da Costa et al., 2000), which had suggested targeting stress early in pregnancy may have implications for reducing symptoms of postnatal depression. These findings indicate the importance of advocating for intervention to include a focus on fostering coping. This is of particular importance when considering the large body of evidence linking maternal stress and stress physiology with poorer infant outcomes (Davis et al., 2011; Diego et al., 2004; Diego et al., 2005; Huizink, Robles de Medina, Mulder, Visser & Buitelaar, 2003). However, the current findings are in contrast to some previous research that coping strategies reduced over time and had little effect on moderating distress during pregnancy (Yali & Lobel, 2002).
Regarding the positive associations between maternal cortisol and pregnancy-related anxiety relating to child health and maternal appearance, there was an association, whereas previously only marginally significant associations have been found (Rothenberger, Moehler, Reck & Resch, 2011). Regarding patterns of symptoms across pregnancy, pregnancy-related anxiety, specifically that related to fear of giving birth, peaked during the third trimester of pregnancy. This finding is consistent with Rothenberger and colleagues (2011) recent study. A trend towards significance was noted for fear for child health, whereby mothers reported greater anxiety for their baby’s well-being during the first trimester of pregnancy than they did later in pregnancy. However, despite their concerns early in pregnancy, mothers’ cortisol levels were at their lowest during the first trimester of pregnancy.

In the multivariate model, cortisol during the second trimester of pregnancy accounted for almost a quarter of the variance in symptoms of anxiety during the third trimester of pregnancy. However, reliability of the anxiety measure was at its lowest during the third trimester. Although the findings of this study supported Huizink’s findings that pregnancy-related anxiety is a discrete form of anxiety, the underlying causes are still not clear. Pregnant women are conspicuous from many other groups in that they carry with them a salient cue. These unique biological cues and the increasingly salient, visual cue as their pregnancy progresses, often dominate the thoughts of the individual and, frequently, the conversation of those who enter their environment. Therefore, it seems possible that when they experience arousal associated with increased cortisol levels they look to their environment to determine the source of anxiety and attribute it to the most salient cue in their environment: pregnancy.

Many of the symptom-related anxiety questionnaires (including the DASS used in this study) may have contributed to a lack of coherent findings in this area of
research, and as such, may need to be supplemented with pregnancy-related anxiety measures when studying anxiety during pregnancy. That is, some of the items on general anxiety measures actually constitute symptoms of pregnancy (e.g., dryness of mouth, breathlessness) or symptoms of arousal such as those associated with high levels of cortisol, which may lead to inaccurate or misleading results. If the pregnancy salience hypothesis proposed is accurate, the pregnancy-related anxiety questionnaires may more accurately capture the individuals’ interpretation of the sensations associated with physiological responses and therefore may more accurately capture anxiety during pregnancy rather than pregnancy-specific anxiety. Post hoc analysis was conducted to remove the physiological component of the anxiety subscale which improved the reliability of the questionnaire to an acceptable level. When cortisol in the second trimester was regressed on anxiety during the third trimester the relationship reduced to exactly that of the pregnancy-related anxiety subscale. It is possible therefore, that the previous findings, whereby cortisol stress responses during the second trimester accounted for one quarter of anxiety during the third trimester of pregnancy, are misinformed; what higher cortisol was actually associated with was the expected physiological responses that one would experience with increased cortisol levels. Further research in a larger study would be required to determine the pathways through which these mechanisms become problematic. This is particularly important given the longer-term outcomes in infants and children that have been noted in previous studies as a result of higher levels of pregnancy-related anxiety (e.g., poorer mental and motor development).

No previous study could be located that has examined cortisol, coping, stress, depression and anxiety during each trimester of pregnancy. Findings relating to low levels of cortisol during the first trimester of pregnancy, despite the presence of
stressors, were unexpected. These findings were inconsistent with previous findings from non-human primate studies. If replicated these findings may indicate specifically human patterns of prenatal development whereby the developing foetus is protected from maternal stress while at its most vulnerable. Consistent with the endocrine patterns of cortisol during pregnancy, maternal cortisol levels were higher in the second and third trimesters of pregnancy.

Limitations

This study has several limitations that the reader must be mindful of in interpreting the results. Firstly, the sample size was not large, and may have limited the statistical power available to assess weaker relationships among some of the key variables. Therefore replication of these findings in a larger, more heterogenous sample, would increase confidence in the present study findings. In addition, variables that could be confounders, such as SES, maternal health, exercise and time since last sleep, were not controlled for statistically. This is a limitation of the present study and future research should consider such confounders given evidence of their importance to understanding associations tested here (e.g., Davis, et al., 2007; Egliston, McMahon & Austin, 2007; Scher, Hall, Zaidman-Zait & Weinberg, 2010). Despite this, important patterns between maternal coping and cortisol levels during pregnancy were demonstrated. Secondly, the measurement of anxiety, depression and stress in mothers was based solely on self-report. Self-report measures are reliable and valid methods to use in the assessment of these constructs, but do not correlate perfectly with clinician-rated measures. Third, no non-pregnancy cortisol levels were collected, which might have revealed differences relevant for understanding anxiety and depressive symptoms.

Future Directions
Consistent with the current study, future research on stress, cortisol and mental health during and after pregnancy should include coping styles to further delineate the role of specific styles of coping, particularly those related to stopping unpleasant thoughts, and pregnancy-related anxiety. Research related to pregnancy-related anxiety, worry and coping likely offer some useful clinical information. However, the link between endocrine changes during pregnancy and onset of anxiety is strong and this too must be addressed. While obtaining saliva samples may allow for identification of women at risk, individual differences in cortisol levels and costs currently render this an unlikely option. Still there remains the need for intervention once dyads at risk have been identified. Clinical Practice Guidelines issued in Australia (Austin, Highet & Guideline Expert Advisory Committee, 2011) recommend cognitive behavioural therapy, interpersonal therapy and psychodynamic therapy as efficacious treatments for women suffering from mild to moderate depression in the postnatal period. Nevertheless, the last decade has also seen a rapid increase in the volume of literature including mindfulness-based therapies. An Australian-based study found mindfulness-based cognitive therapy to be an effective treatment for treatment-resistant depression (i.e., participants having previously tried antidepressant medication and traditional CBT; Kenny & Williams, 2007). Mindfulness characteristics have also been associated with marital satisfaction (Burpee & Langer, 2005), and mindfulness groups were found to be successful in proceeding through grieving processes and emotional well-being in chronic pain patients (Sagula & Rice, 2004). A recent pilot study showed promising results for the use of mindfulness-based interventions in the reduction of pregnancy-related anxiety, depression and negative affect during pregnancy (Duncan & Bardacke, 2010). As one of the principal tools of mindfulness is non-reactivity, this therapy may provide useful techniques to assist with distress caused by physiological changes during
pregnancy. Similarly, as maternal mental health, marital problems and adjustment difficulties are among the most common presenting problems in the postpartum period, mindfulness-based interventions may offer an additional additive treatment to those already provided. Further research in this area with pregnant women, specifically randomised studies may also assist to delineate the roles that reactivity to physiological changes and cognitive rumination play in the onset and maintenance of prenatal mental health problems. This may be particularly useful given that complications in treatment of prenatal anxiety and depression often arise due to non-compliance or discontinuation of medication due to concerns relating to foetal development or the infant in breastfeeding mothers.
CHAPTER 4

Study 1B: Prospective Correlates of Postnatal Depression Symptoms in First Time Mothers: Cortisol, Daily Stress and Anxiety

Due to the importance of decreasing the rate of postnatal depression (PND) to preserve and improve parent and child health, early identification of women at risk for PND has been a growing interest of research (Diego, Field & Hernandez-Reif, 2005; Huizink, Robles de Medina, Mulder, Visser & Buitelaar, 2002). Some of this research has focused on women’s mental health during pregnancy, finding that anxiety and depression in the prenatal period have significant negative impacts on mothers’ mental health and well-being postbirth (e.g., Austin, Tully & Parker, 2007; Bonari et al., 2004; Milgrom et al., 2008). However there is a need for more studies using a prospective design because very few have completed a first assessment of risk factors for PND in the first trimester of pregnancy.

A range of pre-pregnancy stress and mental health factors have been identified as risk factors for PND. For example, depression during the second trimester has been associated with anxiety later in pregnancy, which in turn has been associated with PND (Skouteris, Wertheim, Rallis, Milgrom & Paxton, 2009). However, unanswered questions remain regarding whether maternal mental health problems both early and later in pregnancy are associated with PND, and when normal endocrine processes related to stress, such as cortisol levels, can be indicative of maternal postpartum functioning. To address these issues, the aims in the current prospective study were to examine how cortisol, daily stress, general and pregnancy-related anxiety, and depression measured in each trimester of pregnancy, and the patterns of stress and mental health symptoms over time, were associated with PND symptoms two months after giving birth to a first child.
Stress and Depression

Stress. Stressful life events precede the majority of depressive episodes (Hammen, 2005). Most studies have focussed on the occurrence of stressful events reported retrospectively several months preceding a depressive episode, despite the onset of most episodes occurring within 3 - 4 weeks of an event. This has led to criticism that cognitive biases symptomatic of depressive episodes may affect reporting of events (Hammen, 2005). Studies with retrospective designs have frequently shown error in participants’ reporting including underestimation of mental health symptoms (an der Heiden & Krumm, 1991) and infections during pregnancy (Voldsgaard et al., 2002) when compared to reporting of events closer in time. This highlights the importance of a longitudinal, prospective design that can identify and examine changes over time.

Stress, cortisol, and depression. Pregnancy, childbirth, and the transition to parenting are recognised as stressful life events for many women and difficulties transitioning to these new roles may be further exacerbated by ongoing stress and depressive symptoms. Meta-analysis has indicated that depression rates during the first trimester of pregnancy are consistent with rates of depression in the general female population (Bennett, Einarson, Taddio, Koren & Einarson, 2004). However, these rates almost double as pregnancy progresses through the second and third trimesters. Importantly, many of the studies included in the meta-analysis had excluded women who had experienced recent mental health problems. This likely indicates that these findings are a conservative estimate given that prior history of mental health problems is consistently cited as a strong predictor of prenatal mental health outcomes.

Additionally, endocrine changes associated with the progression of pregnancy may be a correlate of depressed mood. One hormone identified in this process is
cortisol, the hormonal end product of the Hypothalamic Pituitary Adrenal (HPA) axis. Increased cortisol levels have been shown in response to psychosocial stressors (Nierop, Bratsikas, Zimmermann & Ehlert, 2006). However, cortisol levels naturally increase over the course of pregnancy due to complex hormonal processes occurring between mother, placenta and foetus. These processes can result in discrete periods of hypercortisolism with hormone levels similar to those seen in people with Cushing’s Syndrome and major depressive disorder (Kammerer, Taylor & Glover, 2006; Van den Berg, Van Calster, Smits, Van Huffel & Lagae, 2008). Cortisol is such an important marker for stress that developmental psychologists have called for inclusion of such measures to supplement other assessments of stress (e.g., Spangler & Grossmann, 1993).

Increases in prenatal cortisol may assist to identify those at risk of chronic or escalating mental health problems. In one study, higher levels of cortisol reactivity to psychosocial stressors during the second trimester of pregnancy were associated with more PND symptoms two weeks after birth in women with an absence of previous physical or psychiatric disorders (Nierop et al., 2006). During the third trimester of pregnancy, higher cortisol levels in the afternoon and evening have been associated with stress (Obel et al., 2005) and anxiety (Kivlighan, DiPietro, Costigan & Laudenslager, 2008). However, although cortisol increases throughout pregnancy, it is thought that reactivity to stressors, while still present, is reduced during pregnancy (de Weerth & Buitelaar, 2005). While Kivlighan and colleagues measured data very late in pregnancy (36 weeks gestation) other research groups have measured cortisol at two time points during pregnancy (e.g., Nierop et al., 2006; Pleuss, Bolten, Pirke & Hellhammer, 2010). However, no studies could be found that measured cortisol and self-report of stress in
each trimester of pregnancy in order to examine stress and cortisol early and later in pregnancy as correlates of PND.

**Other Correlates of PND: Mothers’ Prenatal Anxiety**

Anxiety during pregnancy is also a risk factor for PND. Support has been found for a bi-directional model of anxiety and depression during pregnancy. Higher depression scores during the second trimester of pregnancy have been shown to predict higher trait-anxiety during the third trimester of pregnancy, which in turn was associated with higher levels of depression in the postbirth period (Skouteris et al., 2009). Importantly, these results remained significant even when those with clinical levels of anxiety and depression were removed from analyses, highlighting the importance of acknowledging subclinical levels of mental health problems during pregnancy. Similarly, other researchers found women high in trait-anxiety during the third trimester of pregnancy had more symptoms of PND eight weeks after birth than women who reported lower scores of trait-anxiety (Austin et al., 2007). However this relationship was no longer significant when confounding variables such as previous episodes of depression, age, and marital status were controlled for. The researchers found that women who reported higher levels of the cognitive component of worry during the third trimester of their pregnancy were 2.6 times more likely to have symptoms of PND after birth than women who reported lower levels of worry, even after controlling for confounding variables.

Other researchers have also suggested the importance of examining different types of anxiety in the onset of PND. Generalised anxiety disorder, characterised by debilitating worry, has predicted symptoms of PND from as early as two weeks after birth until two years after birth, independently of other types of anxiety (Coelho, Murray, Royal-Lawson & Cooper, 2011). More specifically, Huizink and colleagues
(2004) found that pregnancy-related anxiety was a unique form of anxiety that contributed to poorer maternal mental health and infant outcomes (Huizink, Mulder, Robles de Medina, Visser & Buitelaar, 2004). They reported that pregnancy-related anxiety was independent of trait-anxiety late in pregnancy indicating that understanding its three components, namely fear of giving birth, fear of bearing a physically or mentally handicapped child, and concern about one’s appearance may become more important as the pregnancy progresses. This research indicated that the cognitive component of worry may play an important role, or be an important risk marker, for PND.

Combined, these findings suggest complex relationships between cortisol, stress, depression, anxiety symptoms, and pregnancy-related anxiety throughout a discrete time period. However, the paths through which these variables become risk factors for PND is unclear. Further, the timing and chronicity of these factors require further delineation, to best identify women at risk. Therefore a model was also tested to determine the path through which these variables were associated with PND.

**The Current Study Aims and Hypotheses**

In summary, the present study was founded in a multidimensional model of prenatal stress important to the development of PND. This model recognised the importance of cortisol, daily stress, and maternal mental health during pregnancy. Hence, this study examined 1) the occurrence of stressful events, 2) self-appraisal of the distress caused by each event, 3) mental health outcomes that have been linked with stress, including depressive symptoms and anxiety, and 4) a physiological indicator of psychological stress (i.e., cortisol). The aim was to determine whether cortisol, daily stress, general anxiety and depressive symptoms, and pregnancy-related anxiety measured in each trimester of pregnancy were related to symptoms of PND two months
after birth. Once significant pregnancy correlates of PND were identified using correlation; multiple regressions, growth curve modelling, and structural equation modelling (SEM) were used to examine whether 1) measures of cortisol, depressive symptoms, stress and anxiety were associated with PND symptoms (regressions), 2) patterns of change in measures across the three trimesters were associated with PND symptoms (growth curve modelling), and 3) whether there were more complex links between variables that suggested indirect and direct correlations of cortisol, depressive symptoms, stress and anxiety with PND symptoms (SEM). In particular, consistent with evidence that anxiety in trimester 3 of pregnancy is the most proximal risk factor for PND symptoms, a model was tested to determine whether it was a mediator linking prenatal daily stress, cortisol and depressive symptoms with PND.
Method

Participants and Procedure

As reported in Chapter 3, forty-five nulliparous pregnant women were recruited through newspaper advertisements and General Practitioner’s offices. Following an initial screening process, women were eligible to participate in the study if they were between 7 and 13 weeks gestation with no other biological children. Three women were excluded when the gestational age of the foetus was reassessed later in pregnancy indicating that they were not in the first trimester of their pregnancy at commencement of the study and two additional women were excluded because of birth complications. The final participant group comprised 40 women aged between 21 and 42 years, with a mean age of 30.6 years (SD = 5.1 years). The average length of gestation on entry to the study was 9.8 weeks (SD = 2.0). After the initial meeting (T1) data were collected between 21-23 weeks gestation (T2), at 32 weeks gestation (T3) and two months after birth (T4). One participant gave birth prior to completing T3 data and one infant died during the first postbirth week leaving a participant pool of 39. The following measures were administered at each time point.

Measures after Pregnancy Only

Previous research conducted with an Australian sample suggested that the term postpartum depression overlooked “the complexity of psychological distress in the postpartum period” and suggested that screening using the Edinburgh Postnatal Depression Scale (EPDS: Cox, Holden & Sagovsky, 1987) alone may lead to misdiagnoses and subsequently inaccurate treatment (Rowe, Fisher & Loh, 2008; p. 106). Therefore the Depression, Anxiety and Stress Scale (DASS: Lovibond &
Lovebond, 1995) was used to supplement the use of the EPDS in the current study in order to capture a broader range of symptoms.

Postnatal depressive symptoms. Symptoms of postnatal depression were measured using the Edinburgh Postnatal Depression Scale (EPDS: Cox, Holden & Sagovsky, 1987) at T4. The 10-item EPDS was designed as a screening tool to detect postnatal depression in women 6 to 8 weeks postbirth. It contained a stem “Please underline the answer which comes closest to how you have felt in the past 7 days” (e.g., “I have been able to laugh and see the funny side of things”). Responses were scored 0 to 3 according to the severity of symptoms. Items were reverse scored when necessary and summed. Higher scores indicated more severe symptoms. Scores above 9 or endorsement of item 10 (The thought of harming myself has occurred to me) are considered problematic, whereas scores greater than 12 are likely to indicate a depressive illness (Cox et al., 1987). A cut-off of 9 was used for the current study in order to capture a broader range of distress, rather than screen for clinical illness.

Measure used during and after Pregnancy

Depression, anxiety and stress. As reported in Chapter 3, maternal depressive, anxiety and stress symptoms were measured using the Depression Anxiety Stress Scales (DASS; Lovibond & Lovibond, 1995). This brief measure was chosen due to 1) its ability to discriminate between three negative mood states and 2) the availability of Australian norms. The depression subscale consisted of seven items designed to assess negative emotional state (e.g., self-depreciation, anhedonia and lack of interest). The anxiety subscale, designed to measure situational and subjective anxiety including autonomic arousal and muscular effects, consisted of seven items. The remaining seven items formed the stress subscale. These were designed to assess chronic, non-specific arousal (e.g., difficulty relating and getting upset easily). Scores were totalled and
doubled. Higher numbers indicated higher levels of negative affect, anxiety and stress. Cronbach’s α's ranged from .70 to .80 for depression, .53 to .72 for anxiety, and .77 to .85 for stress during pregnancy and .80 for depression, .88 for anxiety, and .84 for stress after birth.

**Measures during Pregnancy**

Cortisol, stress, depressive and general anxiety symptoms and pregnancy-related anxiety were assessed in each trimester. The protocol followed and measures completed were reported in Chapter 3 and comprised:

**Maternal cortisol.** Maternal saliva samples were taken in the afternoon between 4 and 5 pm on 2 consecutive days. Sampling was taken in the afternoon to minimise the effects of circadian variations (de Weerth & Buitelaar, 2005). When possible, and in every case during trimester 2 and 3, participants were sent a text message at approximately 4pm reminding them to produce a sample on the days scheduled for saliva collection. Most participants responded by text on completion of sample collection. Participants were asked to report if they were unwell or using medication at each stage of data collection. Three participant’s samples were postponed by one week due to head colds or flu symptoms. All participants were asked to postpone collection for 24 hours if they had slept during the afternoon on the day collection was due. Participants were asked to produce a saliva sample into a vial no less than 20 minutes after eating or drinking. Samples were frozen at -20°C until analyses were performed. All samples from the same research participant were run, in duplicate, in the same assay using Salimetrics salivary cortisol enzyme immunoassay kit.

**Daily stress.** Perceived stress was measured using the 58-item Daily Stress Inventory (DSI: Brantley, Waggoner, Jones & Rappaport, 1987). The DSI is a self-report measure designed to measure life events during the 24 hours prior to
administration of the questionnaire, and rated the respondent’s subjective stress of these events. Three scores were calculated for each participant: 1) frequency (FREQ) scores were calculated by summing the frequency of life events reported; 2) sum (SUM) scores were calculated by summing the individuals’ rating of subjective distress; and 3) Average Impact Rating (AIR) of the events was calculated by dividing the SUM by the FREQ. Cronbach’s α’s ranged from .92 to .95 for FREQ scores and .91 to .96 for SUM scores throughout pregnancy.

**Pregnancy-related anxiety.** Pregnancy-related anxiety was measured using the short form of the Pregnancy Related Anxiety Questionnaire-Revised (PRAQ-R; Huizink, 2000). This 10-item, self-report measure, consisted of three subscales designed to measure: fear of giving birth, fear of bearing a physically or mentally handicapped child, and concern about one’s appearance. Response options for each item ranged from 1 (absolutely not relevant) to 5 (very relevant). Items on each subscale were summed with higher scores indicating higher levels of pregnancy related anxiety. Across T1, T2 and T3, Cronbach’s α’s of .82 to .84 were found for fear of giving birth, .86 to .91 for fear of bearing a physically or mentally handicapped child, and .87 to .90 for concern about one’s appearance throughout pregnancy.
Results

Descriptive Statistics of Participants and Measures

Examination of T4 data indicated that 11 (28%) of the 39 participants had EPDS scores greater than or equal to the cut-off score of 9 with 7 of these participants scoring greater than or equal to 12, two months after birth. DASS subscale scores indicated seven women reported mild or more severe depressive symptoms, 13 reported mild or more severe anxiety symptoms and 26 reported mild to severe levels of stress.

Descriptive statistics for measures used during pregnancy are provided in Table 1 (Chapter 3) and descriptive statistics for the EPDS used after pregnancy are provided in Table 7.

Table 7

Participants Descriptive Statistics for the Edinburgh Postnatal Depression Scale

(N=39)

<table>
<thead>
<tr>
<th>Measure</th>
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<th>M</th>
<th>SD</th>
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Stress and Mental Health in Each Trimester Associated with PND Symptoms

Correlations and regression models were initially used to examine associations between stress (cortisol and mothers’ daily stress), mental health measured in each trimester during pregnancy and PND symptoms measured by the EPDS two months after birth. Correlations between all T1, T2 and T3 measures with PND symptoms are shown
Table 8. Although no first trimester measure was associated with PND symptoms, anxiety and stress levels in the second and third trimesters of pregnancy were associated with more symptoms of PND. More specifically, anxiety symptoms, pregnancy-related anxiety (fear for child health and concern about appearance), perception and frequency of daily stress and maternal cortisol during the second trimester of pregnancy (T2) were associated with more PND symptoms. Further, anxiety and perception and frequency of daily stress during the third trimester of pregnancy (T3) were associated with more PND symptoms two months after birth. Depressive symptom level was not associated with PND symptoms.

To further examine the T2 and T3 correlates of PND symptoms, PND symptom score was regressed on T2 and T3 measures of depression, anxiety and symptoms of stress using separate models for T2 and T3 measures. The T2 model approached significance ($R^2 = .19, p = .06$), and general anxiety was significantly associated with more symptoms of PND at two months after birth (see Table 9). In a separate model regressing PND symptoms on T2 measures of pregnancy-related anxiety, the model was significant and pregnancy-related anxiety measures accounted for 34% of the variance in PND symptoms ($R^2 = .34, p = .002$). Fears about child health were significantly associated, and fears about appearance were marginally associated, with more PND symptoms after birth (see Table 10).
Table 8

*Correlations between Mothers’ Mental Health and Stress in Trimesters 1, 2 and 3 with Postnatal Depressive Symptoms Measured by the EPDS (N=39)*

<table>
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<th>Repeated measures during pregnancy</th>
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<td>Fear child health</td>
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<td>Fear appearance</td>
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<td>Daily Stress AIR</td>
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<tr>
<td>Cortisol</td>
<td>.15</td>
</tr>
</tbody>
</table>

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

AIR = Average Impact Rating of Daily Stress
Table 9

_Results of Regressing Postnatal Depressive Symptoms on T2 and T3 DASS measures of Depression, Anxiety and Stress_

<table>
<thead>
<tr>
<th>Independent Variables</th>
<th>B (SE B)</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trimester 2 Regression, ( R^2 = .19 ), ( F (3, 35) = 2.7, p = .06 (N = 39) )</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depression</td>
<td>-.05 (.24)</td>
<td>-.04</td>
</tr>
<tr>
<td>Anxiety</td>
<td>.40 (.17)</td>
<td>.44*</td>
</tr>
<tr>
<td>Stress</td>
<td>.02 (.12)</td>
<td>.04</td>
</tr>
<tr>
<td><strong>Trimester 3 Regression, ( R^2 = .30 ) ( F (3, 34) = 4.90, p = .01 (N = 38) )</strong></td>
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<td></td>
</tr>
<tr>
<td>Depression</td>
<td>-.08 (.23)</td>
<td>-.07</td>
</tr>
<tr>
<td>Anxiety</td>
<td>.74 (.21)</td>
<td>.62**</td>
</tr>
<tr>
<td>Stress</td>
<td>-.06 (.15)</td>
<td>-.09</td>
</tr>
</tbody>
</table>

*p < .05, **p < .01.

In the T3 model of depression, anxiety and stress associations with PND symptoms, 30% of the variance in PND was accounted for by T3 measures \( (R^2 = .30, p = .006; \text{see Table 9}) \), and general anxiety was associated with more PND symptoms. However, when PND symptoms was regressed on Trimester 3 measures of pregnancy-related anxiety, the model was not significant \( (R^2 = .12, p = .217; \text{see Table 10}) \).
Table 10

Results of Regressing Postnatal Depressive Symptoms on T2 and T3 PRAQ measures of Fear of Giving Birth, Fear for Child’s Health, and Fear for Appearance

Depression, Anxiety and Stress

<table>
<thead>
<tr>
<th>Independent Variables</th>
<th>B (SE B)</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trimester 2 Regression, ( R^2 = .34, F (3, 35) = 5.99, p = .00 ) (( N = 39 ))</strong></td>
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<td>Fear of Birth</td>
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<td>-.06</td>
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<tr>
<td>Fear for Child Health</td>
<td>.50 (.18)</td>
<td>.43**</td>
</tr>
<tr>
<td>Fear for Appearance</td>
<td>.43 (.25)</td>
<td>.28a</td>
</tr>
<tr>
<td><strong>Trimester 3 Regression, ( R^2 = .12, F (3, 34) = 1.56, p = .22 ) (( N = 38 ))</strong></td>
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<td></td>
</tr>
<tr>
<td>Fear of Birth</td>
<td>.16 (.26)</td>
<td>.10</td>
</tr>
<tr>
<td>Fear for Child Health</td>
<td>.24 (.23)</td>
<td>.18</td>
</tr>
<tr>
<td>Fear for Appearance</td>
<td>.33 (.28)</td>
<td>.21</td>
</tr>
</tbody>
</table>

\( *p < .05 \). \( **p < .01 \).

Patterns of Symptoms and Stress during Pregnancy as Predictors of PND

An additional study aim was to examine changes in stress and mental health during pregnancy, and to test whether these pregnancy-related patterns were additional correlates of greater PND symptoms two months postbirth. Hierarchical Linear and Nonlinear Modelling software (HLM; Raudenbush, Bryk, Cheong & Congdon, 2004) was used to test changes in measures taken during pregnancy. These unconditional growth curve models provided information about patterns of change over time, on average, and tested whether women exhibited significant differences in change over time (i.e., varying patterns of change). For those measures that showed varying patterns of change, latent growth curve modeling (using AMOS software) was used to test
whether patterns of stress and mental health during pregnancy were associated with PND symptoms.

**Unconditional growth curve models and variation among participants.** Of all measures completed in each trimester of pregnancy, two showed increases from T1 to T3 and one showed a decrease, whereas all other measures did not significantly change from T1 to T3. The three measures that showed changes over time were cortisol (slope = .50, \( p < .01 \)), fear of birth (slope = .48, \( p < .05 \)), and fear of child health (slope = -.62, \( p < .05 \)). Hence, cortisol level and fear of birth increased, and fear of child health decreased, from T1 to T3 of pregnancy, on average. Moreover, changes over time varied among the women for three anxiety measures, including general anxiety, fear of birth, and anxiety related to appearance. Therefore, there was only variation to explain for these three measures. Hence, the associations of patterns of change in general anxiety, fear of birth, and anxiety related to appearance with PND symptoms were tested.

**Patterns of mental health and stress during pregnancy as correlates of PND.** The initial levels of general anxiety, fear of birth and anxiety related to appearance (intercept) were each associated with more PND symptoms (all \( p < .05 \)). In particular, women who reported higher levels of anxiety, fear of birth and anxiety related to appearance at T1 (the defined intercept) had more PND symptoms. However, the pattern of change (slope) across pregnancy in each of these measures of anxiety was not associated with symptoms.

For measures that did not show varying prenancy-related patterns among the participants, scores were averaged across the three trimesters and correlations of these “ambient levels” of daily stress or mental health with PND symptoms were tested. Mothers who had higher ambient levels of cortisol (\( r = .33, p < .05 \)) or higher ambient
levels of child health fears \((r = .43, p < .01)\) had more PND symptoms two months after pregnancy. However ambient levels of depressive symptoms and daily stress were not associated with PND symptoms.

**Structural Model of Prenatal Depression, Anxiety, Stress, Cortisol, and PND**

Although examining patterns of change and average levels of anxiety, depression and stress during pregnancy as correlates of PND symptoms identified risk factors for PND, this did not allow for the testing of particular pathways between different measures and how they may provide information about pathways to PND. Previous research supported a model whereby prenatal depression early in pregnancy predicted prenatal anxiety later in pregnancy, which in turn was associated with PND (Skouteris et al., 2009). However, cortisol was not examined in this previous study. Therefore, structural equation modelling (SEM) was used to test the path by which prenatal ambient levels of prenatal stress, depression, anxiety, and cortisol were associated with PND symptoms. In the first model tested, the correlations between prenatal cortisol, depressive symptoms and daily stress, and directional paths of cortisol, daily stress and depression with anxiety were freed. In addition, directional paths from cortisol, daily stress, depression and anxiety to PND symptoms were freed. All non-significant paths were removed resulting in the model shown in Figure 8. Only the correlation between cortisol and daily stress was significant. In addition, depression and cortisol were each directly associated with anxiety \((p < .01)\). Anxiety, in turn, was associated with PND symptoms. There were no significant direct associations of cortisol, depression or daily stress with PND symptoms. This model accounted for 23\% of the variance in PND symptoms and 40\% of the variance in prenatal anxiety. This model fit the data well on all indices, \(\chi^2(2) = 2.47, p = .29\), CFI = .98, RMSEA = .07 \(p = .331\).
A further three models were tested, whereby the measure of anxiety symptom level was replaced by fear for child health, fear of birth, and concern for appearance. None of these models accounted for as much variance in PND symptoms as the initial model reported.

**Figure 8.** Results of a path model testing direct and indirect associations of prenatal stress, depression, cortisol and anxiety with postnatal depression (PND) symptoms

*Note.* Ambient scores were averages of T1, T2, and T3 measures. The first number on each path is the unstandardised path coefficient, the number in parentheses on each path is the standardised path coefficient.

*p < .05. **p < .01.*
Discussion

In the present prospective study multiple aspects of women's well-being during pregnancy were examined as correlates of their symptoms of postnatal depression (PND) measured two months after birth. These aims were based on a model of stress and mental health, which identified daily stress, physiological stress (i.e., cortisol), and mental health factors that have been associated with stress (depression and anxiety) as important correlates of PND symptoms. Patterns of change in cortisol, stress and mental health during pregnancy were also examined as potential correlates of PND symptoms two months after birth.

Maternal Stress and Anxiety during Pregnancy as Correlates of PND

In general, associations of stress and anxiety with PND symptoms were most prominent when measures were taken in the second and third trimesters of pregnancy. However, there was also some indication that first trimester measures are important correlates of PND symptoms. There were many associations of cortisol, stress, anxiety symptoms, and pregnancy-related anxiety with PND symptoms when the measures of stress and symptoms were taken in trimester 2 (T2) or trimester 3 (T3). More specifically, higher maternal cortisol levels, more symptoms of anxiety, more child health and appearance fears, and more frequent and distressing daily stressors reported during the second trimester of pregnancy were related to more symptoms of PND. However, in trimester 3 of pregnancy fewer associations with PND were found; anxiety, frequency of stressors and intensity of distress caused by daily stressors were related to PND symptoms. In simple correlations, no significant relationships were found between trimester 1 (T1) cortisol, mental health symptoms and PND symptoms. However, in growth curve analysis, higher intercepts of general anxiety, and two measures of pregnancy-related anxiety (fear of birth and appearance fears), with the intercept
defined as T1, were associated with more PND symptoms. These findings are consistent with other research and more broadly, the substantial body of research linking higher levels of distress and worry during pregnancy with depressive episodes postbirth (i.e., Austin et al., 2007; Da Costa, Larouche, Drista & Brender, 2000; Sutter-Dally, Giaconne-Marcesche, Glatigny-Dallay & Verdoux, 2004).

One unique contribution of this study was the inclusion of measures of cortisol from each trimester of pregnancy as indicators of stress. In simple correlational analysis, stress cortisol measured during the second, but not the third, trimester of pregnancy was associated with PND symptoms. Moreover, latent growth curve analysis indicated that cortisol increased over time, as is widely recognised in previous research (e.g., Mastorakos & Ilias, 2003), and this pattern did not vary significantly among the participants. This pattern of findings prompted post hoc analyses that indicated that when cortisol was averaged across the three trimesters of pregnancy, this ambient level of cortisol correlated with elevated PND symptoms. These findings suggest that higher ambient levels of cortisol across T1, T2 and T3 of pregnancy contribute to a higher vulnerability to PND symptoms. Nevertheless, cognitive, rather than endocrine, processes may best account for distress during the later stages of pregnancy. Also, despite the lack of support for T1 cortisol as a specific correlate of PND, the inclusion of both physiological and self-report measures commencing in the T1 of pregnancy extends previous research.

In Study 1A (see Chapter 3) cortisol level in T2 of pregnancy was associated with mothers’ reports of more anxiety in T3. It was proposed that maternal interpretation of their subjective experience of elevated cortisol (i.e., interpretation of their physical symptoms captured through the assessment of cortisol) was influenced by the salience of pregnancy as it progressed. That is, mothers with higher cortisol levels were
experiencing and perceiving more arousal, which they in turn interpreted as anxiety. Mothers may then have attributed the anxiety to the pregnancy due to the salience of this cue in their environment. Previously Austin and colleagues (2007) suggested the use of questionnaires targeting the cognitive processes of anxious women during pregnancy had more predictive ability for PND than that measuring trait-anxiety. This study adds to the increasing body of evidence suggesting that accurate understanding, diagnosis and treatment of anxiety during pregnancy is as important as focussing attention on prenatal depressive symptoms to reduce risk for PND and infant difficulties associated with maternal depression early in life.

**Depressive Symptoms during Pregnancy and PND**

Of note, symptoms of depression during pregnancy were not related to PND in the current study. This was true when simple associations and growth curves of depressive symptoms were examined. These findings relating to prenatal and postnatal depressive symptoms are inconsistent with previous research, which has often shown associations of depressive symptoms during pregnancy with PND. The answers to this unexpected finding may best be explained by the homogenous sample characteristics. The sample consisted of predominantly well-educated, middle class, career women in stable relationships. Examination of mean levels of depression throughout pregnancy indicated, at worst, moderate symptoms. However, means for symptoms of anxiety (measured on the same scale) were higher in each trimester. Higher levels of depression in the postpartum may reflect adjustment difficulties as first time mothers adapted to their new roles as mothers, loss of identity within the workplace and reduced income.

**Pregnancy-related Anxiety**

Adding weight to Huizink and colleagues (2004) argument, the findings of the current study indicate that pregnancy-related anxiety contributes to maternal distress
above and beyond symptoms of general anxiety. Pregnancy-related anxiety during T2 of pregnancy accounted for approximately one third of the variance in symptoms of PND when measured two months after giving birth. More specifically, pregnant women's greater child health fear, especially in the second trimester, was associated with their reports of elevated PND symptoms. Although child health fears declined from trimester 1 to trimester 3 on average for women in this study, women who reported higher levels of accumulative fear about their child’s health throughout pregnancy reported more symptoms of PND. This suggests pregnancy-related anxiety scores tap the active, and important, cognitive component of worry during pregnancy that more general measures may not capture. As suggested by the findings of increases in birth and child health fears from trimester 1 to trimester 3, it seems that ruminative processes become increasingly focussed on pregnancy and the foetus as the pregnancy progresses. Of note, also, was the association between more concerns relating to one’s appearance during the second trimester and elevated PND. This finding may indicate the role of self esteem and changing self-concept in PND, which would be a useful area to extend upon in future research.

Limitations

The findings of this study suggest additional new directions for research. First, a much larger study could be conducted with a heterogenous sample. The current study had a modest sample size. Although the sample size was adequate to detect several important relationships between the measured variables, it is possible that low statistical power may have obscured other weaker relationships, such as patterns of change over time. In addition, variables that could be confounders, such as SES, maternal health, exercise, breastfeeding and time since last sleep, were not controlled for statistically. This is a limitation of the present study and future research should consider such
confounders given evidence of their importance to understanding associations tested here (e.g., Stuebe, Grewen, Pedersen, Propper & Meltzer-Brody, 2012). As such, it is important that future research attempt to replicate these significant findings, but also to assess for the presence of other relationships using a larger sample size.

Future research could include multiple mental health measures. In the present study, anxiety, depression, and stress symptoms were all measured using self-report questionnaires. Although all questionnaires were selected because of their demonstrated reliability and validity and each has been associated with clinically diagnosed mental health problems (e.g., Lovibond & Lovibond, 1995), the addition of a clinical interview to assess maternal mental health problems could extend the current study findings. Failure to assess PND symptoms using the EPDS throughout pregnancy, as well as in the postnatal period, was a limitation of the current study. However, a strength was the inclusion of multiple measures of anxiety, both general and specific to pregnancy, and the inclusion of multiple measures of stress, both self-reported and assessed via cortisol assays.

Conclusions

In conclusion, general measures of prenatal anxiety, and worry, measured by pregnancy specific questionnaires, were associated with PND symptoms. Moreover, the initial trajectory levels of anxiety, as well as the average level of prenatal mental health symptoms and cortisol, across three trimesters were also identified as risk factors for PND. Finally, path models suggested that it is general prenatal physiological arousal/anxiety, more so than specific pregnancy-related worries, that is the most proximal risk factor for PND. With associations of average prenatal cortisol and depressive symptom levels with PND fully mediated by anxiety symptoms during pregnancy. Nevertheless, further research to establish a questionnaire measuring both of
these components of arousal would be useful for future research. Future research could expand these findings by conducting a larger prospective study of cortisol, anxiety and stress to further isolate the specific prenatal patterns and pathways associated with PND.
CHAPTER 5

Study 1C: Daily Stress during Pregnancy and Postnatal Mental Health Symptoms are Prospective Correlates of Cortisol Responsivity in Infants

The first year of life is an important time for the development of stress responses and emotional regulation (Larson, White, Cochran, Donzella & Gunnar, 1998; Lewis & Ramsey, 1995). Parenting and maternal well-being play important roles in the development of infants' responses to stress and their capacity to be soothed when distressed during the first year of life. For example, parents who report more emotional withdrawal, such as that associated with depression or avoidance, have been found to have infants with higher levels of baseline cortisol and those who report more use of corporal punishment, have children who have higher cortisol responses to stressors (Bugental, Martorell & Barraza, 2003). Although most research focuses on postnatal maternal and family factors, recent research suggests that prenatal events are also relevant to understanding infants' early stress management and regulatory development (Gutteling, de Weerth & Buitelaar, 2004; Huizink, Robles de Medina, Mulder, Visser & Buitelaar, 2002). How this occurs is still not clear and recent theory points to the mothers' stress hormone cortisol as one factor, which is related to mothers’ stress and mental health during pregnancy. All of these maternal factors can have implications for infants’ later baseline cortisol and cortisol responsivity.

Cortisol is the hormonal end-product of the Hypothalamic Pituitary Adrenal (HPA) axis in humans. This endocrine system is responsible for controlling and regulating the body in response to stress. Cortisol is so integral to this process that stress has been defined as any event that increases levels of cortisol (Heuser & Lammers, 2003). Pregnancy (Mastorakos & Ilias, 2003) and depression (Van den Berg, Van Calster, Smits, Van Huffel & Lagae, 2008) have also been associated with increased
levels of cortisol in adults, and by this definition, suggest that each place stress on the body and impact the HPA axis. Very high levels of cortisol during pregnancy have been associated with slower growth rates in neonates (Diego et al., 2009), low birth weight, and impaired brain development (Diego et al., 2009; Lou et al., 1994). Moreover, mothers’ cortisol levels during pregnancy seem to covary with infant stress response and recovery from stressors shortly after birth (Davis, Glynn, Waffarn & Sandman, 2011).

Although some research has shown links between maternal cortisol during pregnancy and infant outcomes (e.g., Diego et al., 2009), maternal cortisol is often only measured in the second or third trimesters of pregnancy, neglecting the first trimester and the potential importance that elevated cortisol may have in these earliest months of gestation. Studies of non-human primates indicate that maternal stress in early pregnancy has the greatest impact on infant behaviour after birth (e.g., Schneider, Roughton, Koehler & Lubach, 1999). A recent study of human neonates indicated that maternal stress throughout pregnancy, and elevated cortisol early in pregnancy, were each associated with infants’ slower behavioural recovery from the heel-stick procedure (Davis, Glynn, Waffarn & Sandman, 2011). However, data were not collected during the first trimester of pregnancy. The need to more closely examine developmental timing of maternal stress and infant cortisol exposure has been emphasised (Huizink, Robles de Medina, Mulder, Visser & Buitelaar, 2003), but very few human studies have included assessment early enough in pregnancy to compare correlations between first, second, and third trimester maternal cortisol with infant outcomes. Hence, this was one of the aims of the current study, in which maternal stress and cortisol level was examined beginning in the first trimester of pregnancy, a period of rapid cell division and organ development when the foetus is particularly susceptible to environmental experiences and damage (Barker, 1998). Maternal stress was also measured in the
second trimester when the foetal HPA axis develops (Keller-Wood & Wood, 2001; Nader, 2004), and the third trimester when maternal cognitive appraisals of stress may be attenuated (Glynn, Dunkel-Schetter, Wadhwa & Sandman, 2004). After birth, infants’ cortisol response and recovery during a medical stressor (vaccination) were measured within the first four months of life. Infant cortisol reactivity to, and recovery from, vaccination were used as indices of stress regulatory capacity.

Cortisol and Stress during pregnancy

Maternal cortisol levels increase during pregnancy as a result of endocrine processes occurring between the mother, placenta and foetal HPA axes. These changes occur regardless of whether environmental or psychosocial stressors are present, but can also be impacted by stressful events (e.g., Nierop, Bratsikas, Zimmermann & Ehlert, 2006). Early studies examining foetal exposure to maternal cortisol identified that approximately 40% of maternal cortisol passed through the placenta into foetal circulation (Gitau, Cameron, Fisk & Glover, 1998). Maternal cortisol elevations and mental health have been argued to alter development, particularly when they occur over an extended period (e.g., Davis et al., 2011; Diego et al., 2009; Diego, Field, Hernandez-Reif, 2005; Diego, Field, Hernandez-Reif, Cullen, Schanberg & Kuhn, 2004). Hence, although cortisol levels normatively change during pregnancy and are adaptive as responses to stress in the short term, chronic stress may affect the developing foetus and limit the ability to regulate the cortisol response, which is necessary in order to minimise damage to cells (Sapolsky, 1992).

As previous research suggests increased levels of cortisol may be problematic, normal patterns of cortisol throughout pregnancy are important to examine prior to examining the development of infant self-regulatory patterns associated with maternal cortisol and mental health. Mothers’ cortisol levels increase over the course of
pregnancy and this is essential for foetal growth and eventually parturition (Mastorakos & Ilias, 2003). However, from approximately 20 weeks of gestation onwards, as the infant HPA axis develops, corticotrophin releasing hormone (CRH) and subsequently cortisol levels increase dramatically to approximately 2-3 times that of non-pregnant women. Following this peak, cortisol levels begin to fall after 34 weeks gestation with a significant decline towards the end of pregnancy (Mastorakos & Ilias, 2003).

Several studies highlight the importance of these endocrine patterns. For example, women in the second trimester of their pregnancy have demonstrated slower cortisol recovery rates from stressors than those in the third trimester of their pregnancy and non-pregnancy controls. This indicates that when mothers were stressed during this time the foetus was exposed to higher cortisol levels for a longer period of time (Nierop et al., 2006). Additionally, women who showed greater cortisol reactivity to psychosocial stressors during pregnancy also reported more symptoms of postnatal depression (PND) two weeks after birth, even in the absence of previous physical or psychiatric disorders during pregnancy (Nierop et al., 2006). As the foetal HPA axis is developing during the second trimester of pregnancy a priming effect may take place, resulting in a system primed for a stressful environment and unable to regulate the stress response when activated.

However, other researchers examining maternal perception of stress, rather than stress cortisol, have suggested that patterns of stress and anxiety throughout pregnancy, rather than that reported at a discrete period of pregnancy, are associated with poorer outcomes, in this case preterm birth (Glynn, Dunkel-Schetter, Hobel & Sandman, 2008). Another study examining maternal appraisal of stress suggested that stressful events early in pregnancy are perceived as more stressful than those reported later in pregnancy and perception of stress is attenuated during the third trimester (Glynn et al.,
However, these data were not collected systematically throughout each trimester of pregnancy.

**Cortisol, Stress and Infant Outcomes**

An additional aim of this study was to examine maternal stress, measured as daily hassles, during and just after pregnancy as correlates of infants' baseline cortisol and cortisol response and recovery in the first few months of life. Maternal psychological stress and cortisol during pregnancy have also been linked to increased levels of cortisol in children during stressful situations up to five years of age (Gutteling et al., 2004; Gutteling, De Weerth & Buitelaar, 2005), also suggesting that prenatal maternal stress may permanently alter infants' stress responses. In primates, maternal stress during pregnancy has been associated with infants' increased basal levels of cortisol and increased reactivity to psychosocial stress (Clarke, Wittwer, Abbott & Schneider, 1994; O'Connor et al., 2005; Wadhwa, Dunkel-Schetter, Chicz-DeMet, Porto & Sandman, 1996).

Combined, this research suggests that endocrine processes, and psychological responses to these processes (e.g., depression and anxiety), may contribute to infants' cortisol levels, and cortisol response and recovery following stress. Hence, there is a growing body of research recognising the importance of clarifying whether infant cortisol reactions are associated with mothers' cortisol, daily stress and mental health during a particular stage of pregnancy, or whether the accumulative loads of cortisol, perceived stress and mental health throughout pregnancy are the more significant correlates of infants' later cortisol reactions.

**Maternal Mental Health Symptoms**

As maternal mood and anxiety disorders have been linked to poorer infant outcomes, a third aim of the current study was to examine mothers' self-reported
Symptoms of depression and anxiety during and just after pregnancy as correlates of infant cortisol reactions in the first few months after birth. The series of studies by Diego and colleagues (Diego et al., 2005; Diego et al., 2004) provided evidence that the onset and duration of maternal depression affected newborns’ physiology, with earlier onset and longer duration most detrimental to infant cortisol responses and motor movements. Infants born to mothers experiencing depressive symptoms both pre- and post-natally had even less optimal physiological outcomes than infants of mothers who were depressed either pre- or post-natally only. Similar associations between maternal depression and infant physiology and behaviour have also been observed by other researchers (e.g., Dieter, Emory, Johnson & Raynor, 2008; Field, 1995). The ongoing effects of postnatal depression are also recognised as a factor that impacts on the continuing development of infant’s HPA axis as potentially dismissive or intrusive parenting, associated with ongoing maternal depression and anxiety, inhibit the child’s ability to practice effective emotional regulation (Field, 1995).

Other researchers have reported that higher levels of prenatal anxiety, more so than prenatal depression, are associated with lower foetal growth and higher foetal activity at 20-22 weeks gestation (Conde et al., 2010). The authors suggested these findings are important as they indicate potential delays in organisational processes associated with foetal central nervous system development. Therefore, the final aim of the study was to examine maternal mental health symptoms in the postnatal period to examine their role as factors that may potentially modify the development of infant regulation.

The Current Study Aims and Hypotheses

There is accumulating evidence that infant cortisol reactions in the first year of life are outcomes of foetal glucocorticoid exposure and mothers’ experiences of
stressful events, and mental health during or shortly after pregnancy. Yet, the timing of prenatal stress in relation to atypicalities in infant stress responses and basal levels of cortisol have not yet been clearly defined. Huizink et al. (2003) suggested that exposure to stress in early gestation had the most profound effects on development in rhesus monkeys. In humans the foetal HPA axis does not develop until the second trimester of pregnancy (Nader, 2004). This may leave the developing foetus particularly vulnerable to exposure from maternal cortisol during the first trimester of pregnancy as it is during this time that the foetus is most vulnerable to teratogens (Barker, 1998; Barker, 2002). Researchers have called for further investigation into the developmental sequelae of anomalous HPA responsivity in infants. Keenan, Gunthorpe & Grace (2007) suggested, “What needs to be determined is the point in development during which alterations or atypicalities in the stress response system are observed” (p. 135). To date, only one published prospective study examining cortisol and self-reported psychosocial stress of pregnant women commencing in trimester one and continuing across all trimesters of pregnancy and postbirth has been found (e.g., Rothenberger, Resch, Doszpod & Moehler, 2011). The authors reported mothers who perceived less stress and reported less depression during the second trimester had infants with higher behavioural reactivity five months after birth. However, no physiological measures of infant stress were reported.

A multidimensional definition of prenatal stress was used in the current study. This definition recognised the importance of prenatal cortisol levels, daily stress, and maternal mental health symptoms of depression and anxiety. The aim of this study was to determine whether physiological responses to stress (i.e., cortisol), daily stress, general anxiety, and depressive symptoms measured in each trimester of pregnancy were related to infant baseline cortisol and cortisol in response to vaccination two and
four months after birth. In addition the relationship between maternal mental health symptoms two months after birth and infant cortisol reaction and recovery were examined.
Method

Participants and Procedure

As described in Chapters 3 and 4, forty-five pregnant women were recruited through newspaper advertisements and General Practitioners offices. Following an initial screening process, women were eligible to participate in the study if they were between 7 and 13 weeks gestation with no other children. Three women were excluded when the gestational age of the foetus was reassessed later in pregnancy indicating that they were not in the first trimester of their pregnancy at commencement of the study and two women experienced miscarriage. The final participant group comprised 40 women aged between 21 and 42 years, with a mean age of 30.6 years (SD = 5.1 years). The average length of gestation on entry to the study was 9.8 weeks (SD = 2.0). After the initial meeting (T1) data were collected between 21-23 weeks gestation (T2) and at 32 weeks gestation (T3), two months after birth (T4), and 4 months after birth (T5). One participant gave birth prior to completing T3 data. The participant pool delivered 20 male and 20 female infants. One male infant died during the first postbirth week. Thirty-nine mother-infant dyads completed data for the study reported here, however, in some analyses the sample size is smaller due to insufficient volume or deterioration of cortisol samples for infants (e.g., N = 25 and 24 in regressions).

Postbirth Measures

Infant Cortisol. Infant saliva samples were collected, using Salimetrics sorbettes, at 2 and 4 months of age. These were collected when infants were scheduled for immunisation in order to assess infants’ reaction to and recovery from what was expected to be a stressful event. Baseline cortisol levels were obtained from infants 24 hours prior to immunisation and again on the morning of immunisation. Further saliva samples were obtained 20 and 40 minutes after immunisation. Samples were frozen at -
20°C until assayed using Salimetrics salivary cortisol enzyme immunoassay kits according to the manufacturer’s protocol. All samples from the same research participant were analysed in duplicate in the same assay.

To determine infant cortisol reactivity and recovery from the immunisations, two variables were formed. Reactivity was the magnitude of difference between cortisol level measured 20 minutes after the stressor and basal level 24 hours prior to the stressor (i.e., reactivity = 20 minute – baseline). Hence, higher numbers indicated more reactivity. Recovery was the magnitude of difference between cortisol levels taken 20 and 40 minutes after the vaccination (i.e., recovery = 20 minutes – 40 minutes), whereby higher figures indicated more recovery.

**Prebirth Measures**

**Maternal cortisol.** As described in Chapters 3 and 4, in trimesters 1, 2 and 3, in weeks 9-13, 21-23 and 32 of pregnancy, mothers' saliva samples were taken in the afternoon between 4 and 5 pm on 2 consecutive days. Sampling was taken in the afternoon to minimise the effects of circadian variations (de Weerth & Buitelaar, 2005). When possible, and in every case during trimester 2 and 3, participants were sent a text message at approximately 4pm reminding them to produce a sample on the days scheduled for saliva collection. Most participants responded by text on completion of sample collection. Participants were asked to report if they were unwell or using medication at each stage of data collection. Three participant’s samples were postponed by one week due to head colds or flu symptoms. All participants were asked to postpone collection for 24 hours if they had slept during the afternoon on the day collection was due. Participants were asked to produce a saliva sample into a vial no less than 20 minutes after eating or drinking. Samples were frozen at -20°C until assayed, in duplicate, according to the manufacturer’s protocol using Salimetrics salivary cortisol
enzyme immunoassay kits. Baseline levels of cortisol were obtained by averaging the duplicate cortisol levels.

**Postnatal depressive symptoms.** As described in Chapter 4, approximately 8 weeks after giving birth, symptoms of postnatal depression were measured using the Edinburgh Postnatal Depression Scale (EPDS: Cox, Holden & Sagovsky, 1987). The EPDS was designed as a screening tool to detect postnatal depression in women 6 to 8 weeks postbirth. The measure begins with a stem “Please underline the answer which comes closest to how you have felt in the past 7 days” followed by 10 items (e.g., “I have been able to laugh and see the funny side of things”). Responses were scored 0 to 3 according to the severity of symptoms. Items were reverse scored when necessary and summed. Higher scores indicated more severe symptoms. Scores above 9 or endorsement of item 10 (The thought of harming myself has occurred to me) are considered indicative of depression (Cox et al., 1987).

**Daily stress.** As described in Chapters 3 and 4 daily stress was measured in each trimester of pregnancy using the 58-item Daily Stress Inventory (DSI: Brantley, Waggoner, Jones & Rappaport, 1987). The DSI is a self-report measure designed to measure life events during the 24 hours prior to administration of the questionnaire, and rated the respondent’s subjective distress associated with events that occurred. Participants were asked to indicate whether a variety of events had occurred. Sample questions included “Had your sleep disturbed” and “Argued with spouse/boyfriend/girlfriend”. Responses to each item range from 1 (occurred but was not stressful) to 7 (caused me to panic). Response “X” was endorsed if the item did not occur. Three scores were calculated for each participant: 1) frequency (FREQ) scores were calculated by summing the frequency of life events reported; 2) sum (SUM) scores were calculated by summing the individuals’ rating of subjective distress; and 3)
Average Impact Rating (AIR) of the events was calculated by dividing the SUM by the FREQ.

**Depression, anxiety and stress.** As described in Chapters 3 and 4, maternal depressive, anxiety and stress symptoms were measured in each trimester using the Depression Anxiety Stress Scales (DASS; Lovibond & Lovibond, 1995). This brief measure was chosen 1) due to its ability to discriminate between three negative mood states and 2) due to the availability of Australian norms. The depression subscale consisted of seven items designed to assess negative emotional state (e.g., self-depreciation, anhedonia and lack of interest). The anxiety subscale, designed to measure situational and subjective anxiety including autonomic arousal and muscular effects, consisted of seven items. The remaining seven items formed the stress subscale. These were designed to assess chronic, non-specific arousal (e.g., difficulty relating and getting upset easily). Participants indicated how much each statement applied to them during the prior week. Responses ranged from 0 (did not apply to me at all) to 3 (applied to me very much). Scores were totalled and doubled. Higher numbers indicated higher levels of negative affect, anxiety and stress. Cronbach’s α’s ranged from .70 to .80 for depression, .53 to .72 for anxiety, and .77 to .85 for stress during pregnancy and .80 to .78 for depression, .88 to .86 for anxiety, and .84 to .82 for stress after birth.
Results

Descriptive Statistics of Participants and Measures

Descriptive statistics and demographic information for mothers is presented in Table 1, Chapter 3. Two months after birth, of the 39 remaining participants in the study, 23 reported giving birth vaginally (three were forceps assisted) and 16 reported having caesarean section. Participants reported labours ranging from 3 – 39 hours ($M = 13.67, SD = 8.70$). Twenty-five mothers breast-fed and 14 bottle-fed their infants, 10 reported their infants experienced colic. Eighteen participants reported feeling concerned about their infants health after birth, with four reporting ongoing health concerns two months after birth (infants later diagnosed with: vision deficits ($N = 1$); Cerebral Palsy ($N = 1$); multiple health problems due to prematurity ($N = 1$); and Cystic Fibrosis ($N = 1$). Participants estimated they slept between 4 – 9 hours within a 24 hour period. Three participants reported having less support than they needed from their partners (one separated from partner prior to birth).

Means and standard deviations for infant cortisol at T4 and T5 are shown in Table 11. As noted in the Method section two variables were formed to determine infant cortisol reactivity and recovery from the immunisations. Reactivity was the magnitude of difference between cortisol level measured 20 minutes after the stressor and basal level 24 hours prior to the stressor (i.e., reactivity = 20 minute – baseline). Hence, higher numbers indicated more reactivity. Recovery was the magnitude of difference between cortisol levels taken 20 and 40 minutes after the vaccination (i.e., recovery = 20 minutes – 40 minutes), whereby higher figures indicated more recovery. No further computations or transformations were made to the data.

In some analyses the sample size is smaller due to insufficient volume or deterioration of cortisol samples for infants (e.g., $N = 25$ and 24 in regressions).
Independent sample t-tests were conducted to determine whether participants with missing data differed from those with complete cortisol data. There were no significant differences between maternal measures of mental health (DASS), daily stress, pregnancy related anxiety (PRA), coping, cortisol, age, household income or parental education (p > .05).

Table 11

Characteristics of the Infant Sample

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>M</th>
<th>SD</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestation age* (weeks, N = 40)</td>
<td>38.5</td>
<td>2.17</td>
<td>28.75</td>
<td>41.00</td>
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<tr>
<td>Birth Weight (grams, N = 39)</td>
<td>3256</td>
<td>583</td>
<td>1212</td>
<td>4490</td>
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<tr>
<td>Birth Length (cms, N = 37)</td>
<td>50.60</td>
<td>3.77</td>
<td>38.00</td>
<td>56.00</td>
</tr>
<tr>
<td>T4 Baseline Cortisol (µg/L, N = 33)</td>
<td>.16</td>
<td>.22</td>
<td>.02</td>
<td>1.10</td>
</tr>
<tr>
<td>T4 Wake Cortisol (µg/L, N = 31)</td>
<td>.13</td>
<td>.12</td>
<td>.02</td>
<td>.54</td>
</tr>
<tr>
<td>T4 Cortisol Reactivity (µg/L, N = 29)</td>
<td>.10</td>
<td>.35</td>
<td>-.79</td>
<td>.87</td>
</tr>
<tr>
<td>T4 Cortisol Recovery (µg/L, N = 28)</td>
<td>.11</td>
<td>.32</td>
<td>-.38</td>
<td>.96</td>
</tr>
<tr>
<td>T5 Cortisol Baseline (µg/L, N = 35)</td>
<td>.20</td>
<td>.24</td>
<td>.02</td>
<td>1.10</td>
</tr>
<tr>
<td>T5 Wake Cortisol (µg/L, N = 35)</td>
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<td>.29</td>
<td>.01</td>
<td>1.63</td>
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<tr>
<td>T5 Cortisol Reactivity (µg/L, N = 34)</td>
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<td>.25</td>
<td>-.71</td>
<td>.50</td>
</tr>
<tr>
<td>T5 Cortisol Recovery (µg/L, N = 33)</td>
<td>.04</td>
<td>.20</td>
<td>-.74</td>
<td>.52</td>
</tr>
</tbody>
</table>

*Gestational age at birth

Correlations between Mothers’ Cortisol, Stress and Mental Health Symptoms

Pearson's correlations between mothers’ prenatal cortisol, stress, and mental health symptoms are reported in Table 12. There was a moderate relationship between
mothers’ daily stress and cortisol in trimester 2 (T2) of pregnancy. Mothers’ daily stress during T2 was also positively associated with anxiety two months after birth and stress symptoms (measured by the DASS) two and four months after birth. However, these associations were not found when examining mothers’ daily stress (i.e., AIR) during the first or third trimesters.

**Correlations Between Mothers’ Prenatal Cortisol, Daily Stress and Infant Cortisol**

As can be seen in Table 13, Pearson's correlations were also used to examine relationships of maternal baseline cortisol levels and perception of daily stress during each trimester of pregnancy with infant cortisol levels at two and four months of age. Partial correlations were calculated for infant cortisol reactivity and recovery, which controlled for baseline cortisol. In T1, there was a moderate negative relationship between maternal cortisol and infant cortisol reactivity at 4 months of age. No other significant associations were found between mothers' cortisol and infant cortisol at 2 or 4 months. When mothers' daily stressors were examined associations were found for measures in T1 and T3. At T1, mothers who reported more stress had infants with greater cortisol recovery at 2 months (controlling for baseline) but higher baseline at 4 months. At T3, mothers who reported more stress had infants with higher baseline cortisol level at 4 months. Finally, when mothers' reported stress was averaged across the trimesters, a higher "ambient level" of stress was associated with higher infants' baseline cortisol at 4 months.
### Table 12

*Correlations between Mothers’ Prenatal Baseline Cortisol Levels, Daily Stress (AIR), and Postnatal Mental Health Symptoms (N = 39-40)*

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
</tr>
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<tbody>
<tr>
<td>Mother’s Cortisol T1</td>
<td>--</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Mother’s Cortisol T2</td>
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<td></td>
<td></td>
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<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother’s Cortisol T3</td>
<td>.17</td>
<td>.23</td>
<td>--</td>
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<tr>
<td>Mother’s Daily Stress T1</td>
<td>-.18</td>
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<td>-.09</td>
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<td></td>
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<tr>
<td>Mother’s Daily Stress T2</td>
<td>.16</td>
<td>.54**</td>
<td>.15</td>
<td>.35*</td>
<td>--</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Mother’s Daily Stress T3</td>
<td>.07</td>
<td>.30</td>
<td>-.02</td>
<td>.55**</td>
<td>.52**</td>
<td>--</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Mother’s EPDS</td>
<td>.15</td>
<td>.40*</td>
<td>.00</td>
<td>-.03</td>
<td>.45**</td>
<td>.22</td>
<td>--</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother’s Depression 2 month</td>
<td>.01</td>
<td>.13</td>
<td>-.04</td>
<td>-.10</td>
<td>.11</td>
<td>.08</td>
<td>.59**</td>
<td>--</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Mother’s Anxiety 2 month</td>
<td>.18</td>
<td>.21</td>
<td>.02</td>
<td>-.11</td>
<td>.33*</td>
<td>-.01</td>
<td>.64**</td>
<td>.51**</td>
<td>--</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Mother’s Stress 2 month</td>
<td>.21</td>
<td>.11</td>
<td>-.02</td>
<td>.02</td>
<td>.40*</td>
<td>.28</td>
<td>.70**</td>
<td>.69**</td>
<td>.70**</td>
<td>--</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Mother’s Depression 4 month</td>
<td>.30^</td>
<td>.08</td>
<td>.04</td>
<td>-.15</td>
<td>.26</td>
<td>.22</td>
<td>.31</td>
<td>.41**</td>
<td>.18</td>
<td>.20</td>
<td>--</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother’s Anxiety 4 month</td>
<td>.16</td>
<td>.08</td>
<td>-.06</td>
<td>-.25</td>
<td>.15</td>
<td>-.18</td>
<td>.57**</td>
<td>.50**</td>
<td>.87**</td>
<td>.64**</td>
<td>.03</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>Mother’s Stress 4 month</td>
<td>.10</td>
<td>.20</td>
<td>.15</td>
<td>-.11</td>
<td>.50**</td>
<td>.23</td>
<td>.66**</td>
<td>.52**</td>
<td>.60**</td>
<td>.74**</td>
<td>.32*</td>
<td>.62**</td>
<td>--</td>
</tr>
</tbody>
</table>

^p < .10. *p < .05. **p < .01.
Table 13
Correlations between Mothers’ Prenatal Daily Hassles and Cortisol and Infant Cortisol (N = 25 - 35)

<table>
<thead>
<tr>
<th>Infant Cortisol Measures</th>
<th></th>
<th>Mother's Cortisol</th>
<th></th>
<th>Mother's Daily Stress (AIR)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>T1</td>
<td>T2</td>
<td>T3</td>
</tr>
<tr>
<td>2Mth 24 Hour</td>
<td>33</td>
<td>-.10</td>
<td>-.06</td>
<td>-.10</td>
</tr>
<tr>
<td>2Mth Wake</td>
<td>31</td>
<td>.30(^\wedge)</td>
<td>-.20</td>
<td>.07</td>
</tr>
<tr>
<td>2Mth 20 Min (Reactivity)(^a)</td>
<td>25</td>
<td>-.18</td>
<td>.08</td>
<td>.04</td>
</tr>
<tr>
<td>2Mth Recovery Score (20 Min net 40 Min)(^a)</td>
<td>25</td>
<td>-.28</td>
<td>-.06</td>
<td>-.12</td>
</tr>
<tr>
<td>4Mth 24 Hour</td>
<td>35</td>
<td>-.13</td>
<td>-.14</td>
<td>-.19</td>
</tr>
<tr>
<td>4Mth Wake</td>
<td>35</td>
<td>.14</td>
<td>-.12</td>
<td>-.24</td>
</tr>
<tr>
<td>4Mth 20 Min (Reactivity)(^a)</td>
<td>30</td>
<td>-.41(^*)</td>
<td>-.15</td>
<td>-.19</td>
</tr>
<tr>
<td>4Mth Recovery Score (20 Min net 40 Min)(^a)</td>
<td>30</td>
<td>-.18</td>
<td>-.10</td>
<td>-.01</td>
</tr>
</tbody>
</table>

\(^\wedge\)p < .10 \(^*\)p < .05.
Amb = Ambient, the average cortisol or daily stress across the three waves.
\(^a\)Partial correlations controlling for cortisol 24 hours previous.
Mothers' Prenatal Mental Health Symptoms and Infant Cortisol

Correlations were used to examine associations between mothers' mental health symptoms during pregnancy and infants' cortisol when they were 2- and 4-months-old (see Table 14). For these analyses, the focus was on mothers' mental health symptoms across pregnancy. Hence, mothers' accumulative load of depression, anxiety and stress symptoms were calculated as the mean, or “ambient”, levels of each of these scores from the DASS in trimesters 1, 2, and 3. There were no significant associations between ambient levels of prenatal depression, anxiety or stress symptoms and infant cortisol 2 or 4 months after birth.

Maternal Postnatal Mental Health Symptoms and Infant Cortisol

Correlations between maternal postnatal depression symptoms, measured by the EPDS, and depression, anxiety and stress symptoms, measured by the DASS, and infant baseline cortisol, reactivity and recovery are also shown in Table 14. Postnatal depression symptom level (EPDS) was not associated with infant cortisol at 2 or 4 months. Mothers who reported more depression symptoms and stress (measured by the DASS) 2 months after birth had infants with higher morning cortisol levels at 4 months of age. Finally, mothers who reported more depressive symptoms at two months had infants with a lower magnitude of cortisol recovery at two months, indicating less recovery between 20 minutes and 40 minutes after vaccination when controlling for baseline taken 24 hours previously. Corroborating this finding, three other associations between mothers' mental health symptoms and infant cortisol recovery were marginal, p < .10, all indicating that mothers with more mental health symptoms had infants with lower recovery. In particular, mothers who reported more anxiety at 2 months, stress at 2 months, and anxiety at 4 months postbirth had infants with slower recovery at either 2 months or 4 months of age.
Table 14

*Correlations between Mothers’ Mental health Symptoms and Infant Cortisol (N = 24 - 35)*

<table>
<thead>
<tr>
<th>Infant Cortisol Measures</th>
<th>Mothers’ Mental Health Symptoms</th>
<th>2 Mth Prebirth</th>
<th>2 Mth Prebirth</th>
<th>2 Mth Prebirth</th>
<th>Postnatal Dep (EDPS)</th>
<th>Dep, 2mth</th>
<th>Anx, 2mth</th>
<th>Stress, 2mth</th>
<th>Dep, 4mth</th>
<th>Anx, 4mth</th>
<th>Stress, 4mth</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 Mth 24 Hour</td>
<td></td>
<td>33</td>
<td>.01</td>
<td>.01</td>
<td>-.14</td>
<td>-.05</td>
<td>-.09</td>
<td>-.10</td>
<td>.07</td>
<td>.03</td>
<td>-.03</td>
</tr>
<tr>
<td>2 Mth Wake</td>
<td></td>
<td>31</td>
<td>-.22</td>
<td>-.05</td>
<td>-.08</td>
<td>-.02</td>
<td>-.24</td>
<td>-.20</td>
<td>-.13</td>
<td>.02</td>
<td>.09</td>
</tr>
<tr>
<td>2 Mth 20 Min (Reactivity)</td>
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<td>.08</td>
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<td>-.01</td>
<td>.02</td>
<td>-.25</td>
<td>-.09</td>
<td>-.25</td>
<td>-.24</td>
</tr>
<tr>
<td>2 Mth Recovery Score (20 Min net 40 Min)</td>
<td></td>
<td>24</td>
<td>.18</td>
<td>-.06</td>
<td>.12</td>
<td>-.13</td>
<td>-.12</td>
<td>-.38</td>
<td>-.14</td>
<td>-.16</td>
<td>-.38</td>
</tr>
<tr>
<td>4 Mth 24 Hour</td>
<td></td>
<td>35</td>
<td>-.15</td>
<td>.07</td>
<td>-.09</td>
<td>.03</td>
<td>.15</td>
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<td>.29</td>
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<td>4 Mth Wake</td>
<td></td>
<td>35</td>
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<td>-.12</td>
<td>-.10</td>
<td>.16</td>
<td>.44**</td>
<td>.15</td>
<td>.47**</td>
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<td>.03</td>
</tr>
<tr>
<td>4 Mth 20 Min (Reactivity)</td>
<td></td>
<td>29</td>
<td>.12</td>
<td>-.23</td>
<td>-.22</td>
<td>-.22</td>
<td>.04</td>
<td>.01</td>
<td>-.12</td>
<td>.16</td>
<td>-.03</td>
</tr>
<tr>
<td>4 Mth Recovery Score (20 Min net 40 Min)</td>
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<td>29</td>
<td>.25</td>
<td>.22</td>
<td>.03</td>
<td>-.12</td>
<td>-.37</td>
<td>-.05</td>
<td>-.33</td>
<td>.09</td>
<td>-.09</td>
</tr>
</tbody>
</table>

*p < .10. *p < .05.
Dep = depressive symptoms. Anx = anxiety symptoms. Stress = stress symptoms as measured by the DASS.

*Partial correlations controlling for cortisol 24 hours previous.*
Maternal Mental Health as a Moderator of Infant Cortisol from 2 to 4 Months of Age

Multiple regression was used to determine whether maternal mental health symptoms in the postnatal period moderated the relationship between infant cortisol reactivity and recovery between 2 and 4 months of age (see Table 15). Maternal mental health symptoms were the average of depression, anxiety and stress scores from the DASS 2 months postbirth. As recommended, independent variables were centred prior to analysis (Jaccard, Wan & Turrisi, 1990). Moderator (i.e., interaction) effects were formed by multiplying 1) centred values of infant cortisol reactivity by centred total DASS scores, and 2) centred values of infant cortisol recovery by centred DASS scores. Infants’ 4-month cortisol reactivity or recovery was regressed on all independent variables in two separate regression models. One of the two regression models was significant and one significant interaction effect was found indicating that maternal mental health symptoms measured when infants were two months moderated the association between infant cortisol recovery at 2 and 4 months of age.

The significant interaction was examined using simple slopes analysis. High and low scores for infant cortisol recovery and maternal mental health symptoms were calculated as one standard deviation above and below the mean. Results indicated that mothers’ mental health symptoms 2 months postbirth had a stronger negative association with infant cortisol recovery when mental health symptoms were high (i.e., 1 SD above the mean) relative to when they were low (i.e., 1 SD below the mean; see Figure 9). Moreover, infants’ cortisol recovery was lower at 4 months of age when mothers had high relative to low mental health symptoms. Recovery was particularly low in the group of infants who had shown high recovery at 2 months and had mothers with high mental health symptoms 2 months postbirth.
Table 15

Results of Regressing 4-Month (T5) Infant Cortisol Responses on 2-Month (T4) Infant Cortisol Reactivity and Recovery and Maternal Mental Health Symptoms 2 Months Postbirth, and the Moderating Effect of Mental Health Symptoms

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>DV = T5 Infant Reactivity (N = 25)</th>
<th>DV = T5 Infant Recovery (N = 24)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\Delta R^2$</td>
<td>B (SE B)</td>
</tr>
<tr>
<td>Step 1</td>
<td>.09</td>
<td>.15</td>
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<tr>
<td>T4 Mothers’ DASS symptoms</td>
<td>-.002 (.003)</td>
<td>-.12</td>
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<tr>
<td>T4 Infant measure of DV</td>
<td>-.24 (.17)</td>
<td>-.28</td>
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<tr>
<td>Step 2</td>
<td>.10$^\wedge$</td>
<td>.30**</td>
</tr>
<tr>
<td>T4 Mothers’ DASS symptoms (A)</td>
<td>-.004 (.003)</td>
<td>-.23</td>
</tr>
<tr>
<td>T4 Infant measure of DV (B)</td>
<td>-.35 (.18)</td>
<td>-.42*</td>
</tr>
<tr>
<td>A x B</td>
<td>-.03 (.02)</td>
<td>-.35$^\wedge$</td>
</tr>
</tbody>
</table>

DV = dependent variable.

$^\wedge p = .12. ^a p = .08. *p < .05. **p < .01.$
Figure 9. Maternal mental health symptoms 2 months after birth moderates the relationship between infants’ cortisol recovery at 2 and 4 months of age ($N = 24$).
Discussion

In this longitudinal study of mothers and infants, maternal physiological and psychological stress and mental health symptoms during pregnancy were examined as correlates of infant regulatory capacity in response to a vaccination stressor. Additionally, ongoing maternal distress in the postpartum was examined as a moderator of infant cortisol reactivity and recovery between 2 and 4 months of age. No previous study could be located that has examined these factors in a longitudinal design commencing during the first trimester of pregnancy using both physiological and psychological measures of stress. Findings indicated that mothers whose daily stress, but not cortisol, was higher during the first and third trimesters of their pregnancy had infants with higher baseline levels of cortisol at 4 months of age. More reporting of daily stressors during pregnancy may indicate either a more stressful environment, or a tendency to experience events as more stressful, both of which would likely increase in the postpartum as demands increase. As such, these findings may indicate either the infant responding to a stressful environment, or the start of a trend which impacts on mothers’ ability to assist the child to regulate during the first weeks of life.

Examination of associations between maternal cortisol during pregnancy and infant cortisol at four months of age revealed an important relationship. Higher levels of maternal cortisol during the first, but not the second or third, trimester of pregnancy was associated with lower levels of cortisol reactivity in response to a vaccination at age four months. In response to Keenan and colleagues (2007) comment regarding “the point in development during which alterations or atypicalities in the stress response system are observed” our finding may indicate that maternal cortisol impacts an effect on the developing pituitary and adrenal cortex during the first trimester prior to development of the infant HPA axis in the second trimester. Adrenocorticotrophic
hormone (ACTH), a precursor for cortisol production, produced in response to stress can be detected late in the first trimester of pregnancy. However, this relationship between trimester one cortisol and infant cortisol responses was not observed when examining infant cortisol at two months of age indicating that maternal cortisol may predispose the foetus to atypicalities in the stress system which are then shaped in their early environment. Previous researchers have suggested that 3 months of age is a critical period of biobehavioural reorganisation in infants (Emde, Gaensbauer & Harmon, 1976 cited in Gunnar, 2006) and this may partially account for the differences between the 2 and 4 month infant stress reactivity. Gunnar (2006) suggested that attenuation of the infant HPA axis after three months was likely due to either sensitive caregiving, or maturation of the HPA axis’ negative feedback loop (Gunnar, 2006).

The direction of the current findings were not consistent with those found by Clarke and colleagues (1994) when examining nonhuman primates. However, Clarke and colleagues found prenatally stressed primates had higher cortisol across all experimental conditions. An explanation for this may be that the rhesus monkeys were separated from their mothers the day after birth and raised under nursery conditions where they were socialised with other rhesus infants and handled by caregivers for data collection. At one month of age they were moved to small groups with infants from the same prenatal treatment group. Clarke’s finding may reflect development of the infant HPA axis when care giving is suboptimal and opportunities to assist the infants to regulate were absent. Combined with the present findings relating to associations between maternal report of daily stress during pregnancy and higher baseline cortisol in infants, these associations may indicate evidence of the priming effect previously hypothesised (e.g., Huizink, 2003) whereby the foetus was primed for a stressful environment (resulting in higher baseline cortisol) however, as these data suggests,
regulated stress responses more efficiently. This pattern would likely serve to protect the developing brain.

Contrary to previous research no associations between ambient levels of mothers’ mental health symptoms during pregnancy (as measured by the DASS) and infant cortisol were found. In contrast, the current findings supported a model whereby maternal stress (mental health symptoms) during discrete periods of development was associated with atypicalities in HPA axis regulation. Prior research that only focussed on later stages of pregnancy may have missed a critical developmental period through which maternal stress and mental health affects infant development. The data presented here highlights the importance of early detection and treatment of prenatal stress in order to minimise vulnerability throughout infancy. This is particularly important given the suggestion that infant patterns of cortisol responses are set within the first four to six months of age (Lewis & Ramsay, 1995). The findings from the postpartum data further assist our understanding of the importance of these associations.

**Maternal Mental Health Variables Postbirth and Infant Cortisol**

When examining maternal variables in the postnatal period evidence was found to suggest that maternal mental health symptoms are associated with infant regulation (as measured by cortisol) as early as 2 months after birth. Mothers who reported more symptoms of stress and depression measured by the DASS at 2 months had infants with higher baseline cortisol and poorer recovery from the vaccination stressor at four months. However, this relationship was not found when examining symptoms measured by the EPDS. This likely indicates the DASS depression subscale and EPDS are measuring different symptoms and conceptualisations of depressive symptoms and reinforces the need for use of multiple measures of mental health symptoms during the perinatal period. Despite well documented associations between depression and anxiety
(e.g., Lovibond & Lovibond, 1995) these patterns were not as clear when examining postnatal anxiety symptoms, but some marginal associations were found that corroborate the significant associations found for depression and stress. Considering the clinical presentation of depressed mood (flattened affect, emotional withdrawal), stress (increased arousal, irritability, agitation) and anxiety symptoms (uncertainty, reassurance seeking, increased arousal) can help to clarify the slightly stronger associations of maternal depression and stress with infant cortisol when compared to the associations between maternal anxiety and infant cortisol. One possible explanation is that symptoms of maternal depression and stress have more direct implications for infant stress responses because depressed and stressed mothers are more likely to withdraw or become angry or irritated with their infant, whereas anxious mothers may approach and soothe or, when anxiety is overly high, overprotect (Waters, Zimmer-Gembeck & Farrell, 2012).

**Maternal Mental health Symptoms and Changes in Infant Cortisol from 2 to 4 Months**

A composite measure of mothers' mental health was also considered to examine whether mothers' general symptoms of stress, depression and anxiety were associated with changes in infant reactivity and recovery between 2 and 4 months. Findings supported the hypothesis that maternal mental health symptoms are important for understanding infants’ regulatory development during the first 6 months of life. In particular, infants' cortisol recovery was slower at 4 months of age when infants had mothers with high (compared to low) levels of mental health symptoms, and this was especially pronounced among infants who had initially shown high recovery at 2 months. This indicates that maternal mental health symptoms may exert particularly detrimental effects on infant regulatory development when present during periods of
critical reorganisation such as that occurring at approximately three months of age. Previous research has also found associations between maternal mental health symptoms and infant cortisol responses. For example, mental health, emotional withdrawal and maternal depression in the pre and postbirth period has been associated with increased cortisol reactivity in infants (Brennan et al., 2008; Bugental et al., 2003). Whereas higher levels of maternal sensitivity and cooperation have been associated with faster recovery from mild stressors (Albers, Riksen-Walraven, Sweep & de Weerth, 2008) indicating the importance of maternal well-being throughout the entire perinatal period.

**Future Directions**

There are several limitations to this study that could be improved upon in future research. First, the sample of mother-infant dyads was small. Despite this, however, many associations between mothers' cortisol and mental health symptoms and infants' cortisol were found. Hence, the associations that were found were generally moderate in size but many more associations were marginal suggesting that a slightly larger sample size could have revealed additional associations consistent with those reported here. Second, variables that could be confounders, such as SES, maternal health, exercise and time since last sleep, were not controlled for statistically. This is a limitation of the present study and future research should consider such confounders given evidence of their importance to understanding associations tested here (e.g., Davis, et al., 2007; Egliston, McMahon & Austin, 2007; Scher, Hall, Zaidman-Zait & Weinberg, 2010). Third, mothers' mental health symptoms were self-reported. Future research would benefit from the addition of a clinical interview to supplement self-report data. Additionally, the use of the EPDS only after birth rather than at each wave of the study, restricted the analyses that could be done with this measure. Finally, neither the
collection time for wakening cortisol nor vaccination was standardised. This limitation was addressed by using baseline cortisol taken 24 hours prior to the vaccination. This measure was also used when calculating the reactivity and recovery scores used for analyses.

**Conclusions**

A well-defined body of literature examining foetal “programming” of the HPA axis in non-human primates has guided researchers to better understand developmental trajectories, commencing in the prenatal period, associated with infant HPA axis function in humans and the current research extends this work. Prior research has suggested that maternal cortisol early in pregnancy predicts infant behavioural responses to stress whereas maternal cortisol later in pregnancy predicted infant cortisol after birth (Davis et al., 2011). However, associations between the prenatal variables of cortisol and daily stress during the first trimester of pregnancy and infant cortisol at two and four months of age were found. The findings of the current study suggest that cortisol at discrete time points of development affect infant HPA axis regulation and these vulnerabilities are then shaped within the postnatal environment. Consistent with Diego and colleagues research (2004, 2005) it was found that maternal mental health problems after birth had the most detrimental effects on infant self-regulation. The current study data, when collected in trimester 1 of pregnancy, yielded different infant outcomes dependant on maternal physiological stress (i.e., cortisol) and daily psychological stress indicating that maternal mental health appears to have more chronic effects on the development of infant regulation.
CHAPTER 6

Study 1D: Infant Emergent Coping Moderates the Effect of Maternal Mental Health on Infant Cortisol

Infants' quality of social interactions, especially caregiving, during the first year of life shapes the development of language, cognition, stress responses and social and emotional relationships (e.g., Gunnar, 2006; Gunnar & Cheatham, 2003; Gunnar & Donzella, 2002; Gunnar & Quevedo, 2007). Moreover, by 12 months of age, an infant's ability to organise his or her own behaviour around the anticipated responses from the caregiver is thought to be a risk, or protective factor, for later psychopathology (Sroufe & Waters, 1977; Sroufe, Duggal, Weinfield & Carlson, 2000). Maternal mental health in the postnatal period has been identified as a factor that impacts on the quality of caregiving during infancy, and it has been recognised that prenatal experiences also play a role, with these experiences having differential outcomes depending on the quality of care available during the first year of life (Diego, Field, Hernandez-Reif, Cullen, Schanberg & Kuhn, 2004).

Research into the potential role of the prenatal environment on an infant’s development during the early years of life has escalated rapidly in the last decade. Recently such research has focussed on the relationships between maternal mental health and the action of the Hypothalamic Pituitary Adrenal (HPA) axis on infant stress physiology (e.g., Beydoun & Saftlas, 2008; Welberg & Seckl, 2001; Welberg, Seckl & Holmes, 2000) as a function of self-regulatory development (Stansbury & Gunnar, 1994). To date, this research has resulted in mixed findings. For example, some researchers have suggested that suboptimal prenatal and postnatal environments can permanently alter the way an infant physiologically responds to stress, resulting in a stress system that shows regulatory deficits when activated (Diego, Field & Hernandez-Reif, 2005; Diego et al., 2004; Radke-Yarrow, 1991; Cottrell & Seckl, 2009). For example, maternal pregnancy-related anxiety (Tollenaar,
Beijers, Jansen, Riksen-Walraven & De Weerth, 2011) and depression (Field et al., 2010) during pregnancy have each been associated with infant baseline cortisol or cortisol reactivity. Others have suggested that associations found between maternal psychiatric history and higher baseline levels of infant stress hormones may be genetic rather than environmental (Brennan et al., 2008), or report no evidence for a "programming" role of prenatal maternal stress on infant stress physiology (Goedhart et al., 2010). Despite these differences in findings, there is consensus that infants who display early life difficulties with stress reactivity and regulation are at risk for the development of behavioural and emotional problems later in life (see Talge et al., 2007 for review), and this makes it important to continue to address whether maternal factors during and shortly after pregnancy might play a role in infants’ early over-responsiveness to stress in the environment or difficulty down regulating stress reactions once the stressor has subsided.

A well established body of research suggests that infant cognition, language, psychological well-being, and more recently physiological adaptation, are shaped within the context of social relationships (e.g., Gunnar, 2006; Gunnar & Cheatham, 2003; Gunnar & Donzella, 2002; Gunnar & Quevedo, 2007). The quality of the earliest social relationship, that is, the attachment relationship, between a caregiver and infant and the simultaneous development of the infant’s responses to stress were a focus of this study. Commencing data collection in the first trimester of pregnancy, the primary aim was to determine whether a programming effect of maternal cortisol and psychological distress was evident in infant cortisol 12 months after birth. The second aim was to examine the relationships between maternal subjective report of mental health and objective measures of infant behaviour indicative of the quality of attachment relationship, and cortisol responses. To address these aims cortisol was examined following a social stressor at 12 months of age.

**Caregiver-Infant Attachment**
Attachment patterns between a caregiver and an infant develop during the first 12 months of life (Bowlby, 1969; Ainsworth, Blehar, Waters & Wall, 1978). This attachment pattern has been described as an outcome of a history of caregiver-infant interactions during the infant's first year. During this first year the infant draws from previous experiences and outcomes to develop a representation of the caregiver and his or her likely behaviour (Bowlby, 1969), and subsequently organises his or her own behaviour around these particular outcomes (Ainsworth, 1969; Sroufe & Waters, 1977). From the foundation of these interactions, the infant first learns how to cope with stress by relying on the security provided by proximity to a caregiver (Bowlby, 1951), but subsequently develops self-regulatory capacities that supplement the use of others for support (Bowlby, 1951; Carlson & Sroufe, 1995).

In her early work Ainsworth (nee Salter, 1940 as cited in Bretherton, 1992) used the term secure base to refer to the security of optimal caregiving, from which a child may explore and experience the world. Using ethological methods of observation Ainsworth visited 28 Ugandan mother-infant dyads in their homes fortnightly over a 9-month period. Although this study was originally designed to examine infants' responses to weaning and separation from their mothers, observation led to the examination of the processes through which an attachment bond developed. In particular, a major conclusion from this research was that an infant uses his or her mother as a secure base from which to explore the world. Three distinct groups of attachment patterns were observed in infants, distinguishing between those who were securely attached, insecurely attached, and not-yet-attached (Ainsworth, 1967).

In an attempt to replicate and expand these findings, Ainsworth recruited 106 pregnant mothers in Baltimore. After birth, the mother-infant dyads were visited every three weeks for the first year of life in order to determine whether maternal behaviour was
associated with infant development (Bretherton, 1992). As part of this research and based on hundreds of hours of observation, Ainsworth and Wittig (1969) developed the Strange Situation procedure.

The Strange Situation is a reliable and valid laboratory-based assessment of patterns of attachment (Ainsworth et al., 1978). The 8-episode procedure includes two separation and reunion episodes designed to heighten attachment behaviour. Within this context the trained observer may determine individual differences within the attachment relationship with emphasis on how the relationship facilitates regulation of infant emotion and exploration (Carlson & Sroufe, 1995). Specifically, by examining organisation of infant behaviour across the domains of Proximity Seeking, Contact Maintenance, Resistance, and Avoidance the attachment relationship can be classified as anxious-avoidant (A), secure (B), or anxious-resistant (C). The anxious-avoidant classification is characterised by infants who avoid their mothers during the reunion episodes of the Strange Situation, and mothers who tend to be angered and irritated by their infants or rejecting of them. Secure (B) classifications are characterised by infants who exhibit positive behaviours towards their mothers and use them as a secure base from which to explore, and mothers who are sensitive to their infants’ needs. Finally, the anxious-resistant (C) groups are characterised by infants who cry often, and whose anxiety prohibits them from exploration and whose mothers are less responsive to infant signals and crying than mothers of B group infants (Ainsworth et al., 1978). Recognising that a small proportion of infants did not fit into any of these categories, lacking a coherent pattern of attachment behaviours, Main and Solomon (1990) expanded on this research and added a fourth category, disorganised/disoriented (D). Disorganised/disoriented classifications are characterised by infants whose behaviour lacks an observable goal or explanation and parents who exhibit frightened or frightening behaviour such as severe depression, anxiety or contradictory signals (Main & Solomon, 1990).
In recent expansions of Attachment Theory, attachment behaviour is now described as an emerging infant coping strategy that is relied upon to minimise distress (Kobak, Cassidy, Lyons-Ruth & Ziv, 2006.; Sroufe & Waters, 1977). In particular, infants who seek proximity to their caregivers when stress occurs have been found to exhibit less physiological stress reactivity than infants who show other "coping" behaviours, such as anger or avoidance of the caregiver (Hertsgaard, Gunnar, Erickson, & Nachmias, 1995). As the quality of caregiving and the development of infant regulation and HPA development are related, previous research have supported the importance of measures of physiological stress responses (e.g., cortisol) as a way to validate the assessment of caregiver-infant attachment in the Strange Situation (Spangler & Grossmann, 1993).

**Maternal Mental Health, Stress and Attachment Outcomes**

Maternal mental health problems in the postpartum period exacerbate parental stress, and interfere with quality of caregiving necessary for optimal infant outcomes. For example, research suggests that poor maternal mental health negatively impacts on infant physiology, cognition, and emotional competence (e.g., Diego et al., 2004; Murray & Cooper, 1996). Maternal sensitivity is characterised by empathic understanding and responsivity to the infant and these maternal characteristics are necessary to soothe the infant when distressed in the short term (Skinner & Zimmer-Gembeck, 2007; Sroufe, 1996), and are prerequisites for attachment security in the long term (Kobak et al., 2006). Previously, lower levels of maternal sensitivity, associated with depression and anxiety have been found to be detrimental for infant development, including impaired cognitive ability in males (Milgrom, Westley & Gemmill, 2004), and intense infant avoidance has been observed in response to maternal overstimulation and intrusiveness, often observed in anxious mothers (Beebee, 2000).
While pathological parenting (e.g., child abuse) has been associated with the poorest of infant outcomes (e.g., Cicchetti & Toth, 2005), other parenting behaviours, such as smacking and emotional withdrawal, have also been associated with higher basal levels of infant cortisol and higher levels of cortisol reactivity in response to stressors (Bugental, Martorell & Barraza, 2003). Lack of maternal responsivity, such as that related to maternal depression, has also been associated with trends towards infants' poorer regulation of heart rate and negative affect (Haley & Stansbury, 2003). Other researchers have suggested that basal levels of cortisol may be genetically predisposed, whereas infant cortisol reactivity in response to stressors may be a result of exposure to parental depression (Brennan et al., 2008).

These patterns of associations between parenting behaviours associated with mental health problems and infant stress regulatory responses are important as prolonged exposure to glucocorticoids can result in an organism that is acutely prepared for stress with limited ability to regulate the response once activated (Cottrell & Seckl, 2009). This may be particularly true in the first year of human life as the infant relies so heavily on the caregiver to aid in the regulation of stress responses. Researchers have suggested that typical adult cortisol patterns are established sometime between three (Larson, White, Cochran, Donzella & Gunnar, 1998) and six months of age (Lewis & Ramsay, 1995). This likely indicates that a consistent pattern of cortisol reactivity has been established within the first half year of life (Lewis & Ramsay, 1995). It has been suggested that when care is optimal a human infant’s stress system enters a period of hypo-responsivity by the end of the first year that may protect the developing brain (Gunnar, 2006).

Researchers studying whether cortisol reactivity is related to caregiver-infant attachment relationships have found that infants who were prone to distress exhibited greater cortisol responses as a result of the Strange Situation compared to those who showed less
distress (Gunnar, Mangelsdorf, Larson & Hertsgaard, 1989). Spangler and Grossmann (1993) found greater cortisol responses, indicating greater stress responses, in insecure avoidant and disorganised infants compared to securely attached infants. However, these findings were only significant for the disorganised group when compared to the secure group. These findings were replicated, in a high risk sample of 35 infants (Hertsgaard et al., 1995). In this study, infants with disorganised attachment classification had greater cortisol responses during the Strange Situation compared to all other infants.

In the current study, associations of pre- and post-natal maternal measures with infant cortisol and attachment will be examined to address four main aims. The primary aim is to determine whether evidence exists to support the programming hypothesis of infant cortisol. That is, whether maternal prenatal mental health symptoms, cortisol, stress and pregnancy-related anxiety are associated with infant cortisol 12 months after birth. A second aim was to examine associations between maternal daily stress, and postnatal mental health symptoms, with infant baseline cortisol and cortisol responsivity to a stressor. Associations of infant baseline cortisol and cortisol responsivity with infant attachment were examined, as a third aim, given previous evidence that physiological adaptation is shaped within the context of social relationships (e.g., Gunnar, 2006; Gunnar & Cheatham, 2003; Gunnar & Donzella, 2002; Gunnar & Quevedo, 2007). Finally, in Study 1A, mothers’ coping reduced the positive associations between daily stressors with their cortisol levels. Therefore, the fourth aim of the current study was to examine whether similar associations would be found among infants. Hence, infant coping behaviours were observed during a stressful event, the Strange Situation, and infant coping was tested as a potential correlate of reduced infant cortisol reactivity and recovery.

The following hypotheses about the correlates of 12-month-old infant baseline cortisol and cortisol in reaction to a Strange Situation stressor were tested:
1. Maternal daily stress and cortisol measured during pregnancy will be associated with higher infant baseline cortisol at 12 months of age, and less infant cortisol reactivity in response to the Strange Situation at 12 months of age.

2. Maternal report of pregnancy-related anxiety measured during each trimester of pregnancy will be associated with higher infant baseline cortisol at 12 months of age and less infant cortisol reactivity.

3. Higher levels of maternal mental health symptoms postbirth (measured when infants were 2, 4 or 12 months of age) will be associated with infants' higher baseline cortisol at 12 months of age, and less infant cortisol responsivity (i.e., less reactivity and recovery) following the Strange Situation at 12 months of age.

4. Infants with a secure attachment relationship will have lower basal cortisol levels, less cortisol reactivity and cortisol recovery compared to infants with an insecure attachment relationship.

The following hypotheses about the correlates of infants' coping behaviours during the Strange Situation stressor at 12 months were tested:

5. Infant adaptive coping displays of proximity seeking and contact maintenance during the Strange Situation will be associated with less cortisol responsivity (i.e., less cortisol reactivity and recovery).

6. Infant maladaptive coping displays of resistance and avoidance during the Strange Situation will be associated with more cortisol responsivity (i.e., more cortisol reactivity and recovery).

7. Infant adaptive coping displays of proximity seeking and contact maintenance during the Strange Situation will weaken the relationship between maternal mental health symptoms and infant cortisol at baseline as well as reactivity and recovery.
Method

Participants and Procedure

As described in Chapters 3, 4, and 5, forty-five nulliparous pregnant women were recruited through newspaper advertisements and General Practitioners offices. Following an initial screening process, women were eligible to participate in the study if they were between 7 and 13 weeks gestation with no other children. Three women were excluded when the gestational age of the foetus was reassessed later in pregnancy indicating that they were not in the first trimester of their pregnancy at commencement of the study. A further two women experienced miscarriage during the first trimester of pregnancy and their data were subsequently removed prior to analyses. The final participant group comprised 40 women aged between 21 and 42 years, with a mean age of 30.6 years (SD = 5.1 years). The average length of gestation on entry to the study was 9.8 weeks (SD = 2.0). After the initial meeting (T1) data were collected between 21-23 weeks gestation (T2), at 32 weeks gestation (T3), 2 and 4 months after birth (T4 and T5 respectively) and finally 12 months after birth (T6). One participant gave birth prior to completing T3 data. One infant died during the first postbirth week and one participant was unable to be contacted for T6 data collection leaving a participant pool of 38. In some analyses, the sample size is smaller due to insufficient volume or deterioration of saliva supplied in samples from infants (e.g., $N = 25$ and 24 in regressions). The following measures were administered at each time point.

Maternal Measures in Trimesters 1, 2 and 3 of Pregnancy

Maternal cortisol. As reported in Chapters 3, 4, and 5, maternal saliva samples were collected in the afternoon between 4 and 5 pm on 2 consecutive days. Sampling was taken in the afternoon to minimise the effects of circadian variations (de Weerth & Buitelaar, 2005). When possible, and in every case during trimester 2 and 3, participants were sent a text
message at approximately 4pm reminding them to produce a sample on the days scheduled for saliva collection. Most participants responded by text on completion of sample collection. Participants were asked to report if they were unwell or using medication at each stage of data collection. Three participant’s samples were postponed by one week due to head colds or flu symptoms. All participants were asked to postpone collection for 24 hours if they had slept during the afternoon on the day collection was due. Participants were asked to produce a saliva sample no less than 20 minutes after eating or drinking. Samples remained frozen at -20°C until analyses were performed. All samples from the same research participant were run, in duplicate, in the same assay using Salimetrics salivary cortisol enzyme immunoassay kit according to the manufacturer’s protocol.

**Pregnancy-related anxiety.** As reported in Chapters 3 - 5, pregnancy-related anxiety was measured using the Pregnancy Related Anxiety Questionnaire-Revised (PRAQ-R; Huizink, 2000). This 10-item, self-report measure, consisted of three subscales designed to measure: Fear of giving birth, fear of bearing a physically or mentally handicapped child, and concern about one’s appearance. Items on each subscale were summed with higher scores indicating higher levels of pregnancy-related anxiety. Across T1, T2 and T3, Cronbach’s α's ranged from .82 to .84 for fear of giving birth, .86 to .91 for fear for child health, and .87 to .90 for concern about appearance.

**Maternal Pre- and Postbirth Measures**

**Depression, anxiety and stress.** As described in Chapters 3 - 5, depression, anxiety and stress symptoms were measured using the 21-item Depression Anxiety Stress Scales (DASS; Lovibond & Lovibond, 1995). This brief measure was chosen 1) due to its ability to discriminate between three negative mood states and 2) due to the availability of Australian norms. Participants indicated how much each statement applied to them during the prior week. Scores were totalled and doubled. Higher numbers indicated higher levels of negative
affect, anxiety and stress. Cronbach’s $\alpha$'s ranged from .70 to .80 for depression, .53 to .72 for anxiety, and .77 to .85 for stress during pregnancy and .78 - .86; .85-.88; and .82 - .85 respectively for depression, anxiety and stress after birth.

**Daily stress.** As described in Chapters 3 - 5, daily stress was measured in each trimester of pregnancy using the 58-item Daily Stress Inventory (DSI: Brantley, Waggoner, Jones & Rappaport, 1987). The DSI is a self-report measure designed to measure life events during the 24 hours prior to administration of the questionnaire, and rated the respondent’s subjective stress of these events. Participants were asked to indicate whether a variety of events had occurred. Sample questions included “Had your sleep disturbed” and “Argued with spouse/boyfriend/girlfriend”. Responses to each item range from 1 (occurred but was not stressful) to 7 (caused me to panic), response “X” was endorsed if the item did not occur. Three scores were calculated for each participant: 1) frequency (FREQ) scores were calculated by summing the frequency of life events reported; 2) sum (SUM) scores were calculated by summing the individuals’ rating of subjective distress; and 3) Average Impact Rating (AIR) of the events was calculated by dividing the SUM by the FREQ. Cronbach’s $\alpha$'s ranged from .92 to .95 for frequency scores and .91 to .96 for SUM scores throughout pregnancy. Four and twelve months after birth Cronbach’s $\alpha$'s ranged from .90-.96 for SUM and .88-.95 for FREQ.

**Maternal Postbirth Only Measures**

**Postnatal depressive symptoms.** As described in Chapter 4, symptoms of postnatal depression were measured using the Edinburgh Postnatal Depression Scale (EPDS: Cox, Holden & Sagovsky, 1987) at T4. The 10-item EPDS was designed as a screening tool to detect postnatal depression in women 6 to 8 weeks postbirth. It contained a stem “Please underline the answer which comes closest to how you have felt in the past 7 days” (e.g., “I have been able to laugh and see the funny side of things”). Responses were scored 0 to 3
Maternal and Infant Stress

according to the severity of symptoms. Items were reverse scored when necessary and summed. Higher scores indicated more severe symptoms. Scores above 9 or endorsement of item 10 (The thought of harming myself has occurred to me) are considered problematic (Cox et al., 1987).

Infant Measures

Infant Cortisol. As reported in Chapters 4 and 5, infant saliva samples were collected using Salimetrics sorbettes at 2 (T4), 4 (T5) and 12 - 15 months of age (T6). At T4 and T5, baseline cortisol levels were obtained from infants 24 hours prior to immunisation and again on the morning of immunisation. Further saliva samples were obtained 20 and 40 minutes after immunisation. At T6 baseline cortisol samples were collected 24 hours prior to the scheduled time of the Strange Situation and on the morning of the Strange Situation procedure after wakening. Samples were also collected 20 and 40 minutes after completion of the procedure. Samples were frozen at -20°C until assayed using Salimetrics salivary cortisol enzyme immunoassay kit according to the manufacturer’s protocol. All samples from the same research participant were run, in duplicate, in the same assay. Consistent with data collected at T4 and T5 (Chapter 5), two variables were formed to determine infant cortisol reactivity and recovery from the Strange Situation. Reactivity was the magnitude of difference between cortisol level measured 20 minutes after the stressor and basal level 24 hours prior to the stressor (i.e., reactivity = 20 minute – baseline). Hence, higher numbers indicated more reactivity. Recovery was the magnitude of difference between cortisol levels taken 20 and 40 minutes after the Strange Situation (i.e., recovery = 20 minutes – 40 minutes), whereby higher figures indicated more recovery.

Mother-infant attachment. The Strange Situation (Ainsworth et al., 1978), an observational assessment, was used to measure mother-infant attachment and to assess each infant’s coping behaviours. The Strange Situation is a widely used, reliable and valid
laboratory based assessment of patterns of attachment (e.g., Ainsworth et al., 1978, Hertsgaard et al., 1995; Spangler & Grossman, 1993). The 8-episode procedure includes two separation and reunion episodes designed to stress the infant in order to heighten attachment behaviour. By examining organisation of infant behaviour across the domains of proximity seeking, contact maintenance, resistance, and avoidance during the reunion episodes the attachment relationship can be classified as anxious-avoidant (A), secure (B), or anxious-resistant (C), and disorganised (D). Two coders assessed each Strange Situation. Two research assistants coded 50% of the assessments each and the primary researcher coded all assessments. When disagreement of greater than two points occurred on any subscale between coders, and this could not be resolved by rewatching the session, the third coder was asked to assist in resolution of the disagreement. Following discussion with the third coder the primary researcher made the final decision regarding which two sets of codes to record.

Two of the coders had each attended seventy hours training in Attachment Classification at the Institute of Child Development in Minnesota. On completion of training they completed the Attachment Reliability Test. This test relates to the attachment training and involved approximately 50 hours of video coding. Both coders were deemed reliable to code the traditional A-B-C classifications (minimum reliability standard is .80). The third coder, had attended 35 hours of training in Attachment Classification however, had not yet completed the Attachment Reliability Test at time of coding the procedures for this study. Prior to coding the data for this study the three coders independently coded 10 practice Strange Situation procedures and achieved minimum reliability of .80 as a research group.

Intraclass reliability scores were calculated for reunion episodes. Average measures Cronbach’s α’s for the episode 5 reunions were .95 for proximity seeking, .99 for contact maintenance, .88 for resistance, and .85 for avoidance. For episode 8 reunions average measures Cronbach’s α’s were .96, .90, .95, and .85 for proximity seeking, contact
maintenance, resistance and avoidance respectively. Intraclass reliability for Attachment classification was .99 indicating near perfect agreement.
Results

Outliers

Examination of distributions of variables and residual plots following regression modelling indicated one infant had a baseline cortisol level >4 SD above the mean of scores for the whole cohort at this time period. To maintain this participant in the study, the baseline cortisol level was set to the score of the next ranking child and reactivity and recovery scores were recalculated. As noted in the Method section two variables were formed to determine infant cortisol reactivity and recovery from the Strange Situation stressor. Reactivity was the magnitude of difference between cortisol level measured 20 minutes after the stressor and basal level 24 hours prior to the stressor (i.e., reactivity = 20 minute – baseline). Hence, higher numbers indicated more reactivity. Recovery was the magnitude of difference between cortisol levels taken 20 and 40 minutes after the Strange Situation (i.e., recovery = 20 minutes – 40 minutes), whereby higher figures indicated more recovery. Means, standard deviations, minimum and maximum levels of cortisol are reported in Table 16.

In some analyses the sample size is smaller due to insufficient volume or deterioration of cortisol samples for infants (e.g., \(N = 25\) and \(24\) in regressions). Independent sample \(t\)-tests were conducted to determine whether participants with missing data differed from those with complete cortisol data. There were no significant differences between maternal measures of mental health (DASS), daily stress, pregnancy related anxiety (PRA), coping, cortisol, age, household income or parental education (\(p > .05\)).

Descriptive Statistics and Demographics

Descriptive statistics for mothers and infants are reported in Table 1 (Chapter 3) and Table 11 (Chapter 5), respectively.
Table 16

Descriptive Statistics for Infant Cortisol at T6

<table>
<thead>
<tr>
<th>Infant Cortisol</th>
<th>M</th>
<th>SD</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
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<tbody>
<tr>
<td>T6 Cortisol Baseline (µg/L, N = 30)</td>
<td>.13</td>
<td>.13</td>
<td>.01</td>
<td>.49</td>
</tr>
<tr>
<td>T6 Wake Cortisol (µg/L, N = 29)</td>
<td>.18</td>
<td>.22</td>
<td>.03</td>
<td>.90</td>
</tr>
<tr>
<td>T6 Cortisol Reactivity (µg/L, N = 29)</td>
<td>-.01</td>
<td>.14</td>
<td>-.36</td>
<td>.20</td>
</tr>
<tr>
<td>T6 Cortisol Recovery (µg/L, N = 33)</td>
<td>.02</td>
<td>.13</td>
<td>-.44</td>
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</tr>
</tbody>
</table>

Hypothesis 1 and 2: Prenatal Maternal Correlates

Correlations were used to examine relationships of infant cortisol at 12 months with mothers’ baseline cortisol, daily stress, pregnancy-related anxiety, and mental health symptoms measured in each trimester of pregnancy (see Table 17, Table 18, and Table 19). In partial support of Hypothesis 1, a moderate to strong association was found between maternal cortisol in T2 and infant baseline cortisol at 12 months (T6). Mothers with higher cortisol levels at T2 had infants with higher baseline levels of cortisol 12 months after birth. However, contrary to Hypothesis 1, there was no relationship between mothers' prenatal report of daily stress (T1-T3) and infant baseline cortisol at 12 months (T6; see Table 17).

Regarding maternal pregnancy-related anxiety, in partial support of Hypothesis 2, mothers with more fears regarding their changing appearance during each trimester of pregnancy had infants with higher baseline cortisol at 12 months of age (see Table 18). Mothers’ fears relating to child health during T2 was also positively associated with infant baseline cortisol.

Hypothesis 3: Postnatal Maternal Correlates
Compared to associations of maternal measures prior to giving birth, more consistent associations were found between infants' cortisol at 12 months and mothers' stress and mental health postbirth. In support of Hypothesis 3, postnatal maternal symptoms of mental health (measured by the DASS) and daily stress were consistently negatively associated with infant cortisol recovery from the Strange Situation stressor at 12 months of age. Mothers who reported more symptoms of depression, anxiety and stress 2 months after birth; anxiety 4 months after birth; and daily stress (AIR), depression and anxiety 12 months after birth had infants with poorer cortisol recovery. However, only maternal depression 12 months after birth was associated with infant baseline cortisol (see Table 19).
Table 17

Correlations between Mother’s Prenatal Cortisol and Perceived Stress and Infant Cortisol at 12 Months

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>1</th>
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<td>Mother’s Cortisol T1</td>
<td>40</td>
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<td>Mother’s Cortisol T2</td>
<td>40</td>
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<td>Mother’s Cortisol T3</td>
<td>39</td>
<td>.17</td>
<td>.23</td>
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<tr>
<td>Mother’s AIR T1</td>
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<td>.25</td>
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<tr>
<td>Mother’s AIR T2</td>
<td>40</td>
<td>.16</td>
<td>.54**</td>
<td>.14</td>
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<tr>
<td>Mother’s AIR T3</td>
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<td>.51**</td>
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<tr>
<td>Infant 24 hr baseline</td>
<td>30</td>
<td>-.27</td>
<td>.61**</td>
<td>.13</td>
<td>.00</td>
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<td>25</td>
<td>-.16</td>
<td>.25</td>
<td>-.00</td>
<td>.21</td>
<td>-.08</td>
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<td>Infant Recovery^a</td>
<td>24</td>
<td>-.05</td>
<td>-.02</td>
<td>.16</td>
<td>.29</td>
<td>.02</td>
</tr>
</tbody>
</table>

^p < .10. *p < .05. **p < .01.

^a Partial correlations controlling for cortisol 24 hours previous.

AIR = Average Impact Rating of Daily Stress
Table 18

*Correlations between Mother’s Pregnancy-Related Anxiety and Infant Cortisol at 12 Months*

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>1</th>
<th>2</th>
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<th>6</th>
<th>7</th>
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<tr>
<td>1 Fear Birth T1</td>
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<td>2 Fear Child Health T1</td>
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<td>.18</td>
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</tr>
<tr>
<td>3 Fear Appearance T1</td>
<td>40</td>
<td>.54**</td>
<td>.36*</td>
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<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>4 Fear Birth T2</td>
<td>40</td>
<td>.80**</td>
<td>.12</td>
<td>.39*</td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>5 Fear Child Health T2</td>
<td>40</td>
<td>.09</td>
<td>.56**</td>
<td>.33*</td>
<td>.21</td>
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<td>6 Fear Appearance T2</td>
<td>40</td>
<td>.44**</td>
<td>.34*</td>
<td>.82**</td>
<td>.39*</td>
<td>.41**</td>
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<td>7 Fear Birth T3</td>
<td>39</td>
<td>.66**</td>
<td>-.03</td>
<td>.13</td>
<td>.74**</td>
<td>-.02</td>
<td>.05</td>
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<td>8 Fear Child Health T3</td>
<td>39</td>
<td>.18</td>
<td>.50**</td>
<td>.41*</td>
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<td>.67**</td>
<td>.43**</td>
<td>-.05</td>
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<tr>
<td>9 Fear Appearance T3</td>
<td>39</td>
<td>.29</td>
<td>.28^</td>
<td>.64**</td>
<td>.25</td>
<td>.31^</td>
<td>.84**</td>
<td>-.00</td>
<td>.45**</td>
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<tr>
<td>10 Infant 12 Month 24 hr baseline</td>
<td>30</td>
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<td>.24</td>
<td>.36*</td>
<td>-.02</td>
<td>.38*</td>
<td>.49**</td>
<td>-.25</td>
<td>.11</td>
<td>.38*</td>
</tr>
<tr>
<td>11 Infant 12 Month Reactivity^a</td>
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<td>.06</td>
<td>-.11</td>
<td>.28</td>
<td>.05</td>
<td>-.02</td>
<td>.15</td>
<td>.06</td>
<td>-.07</td>
<td>.13</td>
</tr>
<tr>
<td>12 Infant 12 Month Recovery^a</td>
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<td>.32</td>
<td>-.11</td>
<td>.39^</td>
<td>.24</td>
<td>-.22</td>
<td>.18</td>
<td>-.05</td>
<td>-.01</td>
<td>.13</td>
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</tbody>
</table>

^p < .10. *p < or = .05. **p < .01.

^aPartial correlations controlling for cortisol 24 hours previously.
### Table 19

*Correlations between Mother’s Postnatal Mental Health and Stress and Infant Cortisol at 12 Months*

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
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<th>10</th>
<th>11</th>
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<td>34</td>
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<tr>
<td>Depression 2 Months</td>
<td>--</td>
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<td>--</td>
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<tr>
<td>Anxiety 2 Months</td>
<td>.54**</td>
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<td>.70**</td>
<td>.73**</td>
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<td>--</td>
<td>--</td>
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<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Depression 4 Months</td>
<td>.31</td>
<td>.16</td>
<td>.13</td>
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</tr>
<tr>
<td>Anxiety 4 Months</td>
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<td>.89**</td>
<td>.69**</td>
<td>.03</td>
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<td>--</td>
</tr>
<tr>
<td>Stress 4 Months</td>
<td>.52**</td>
<td>.64**</td>
<td>.70**</td>
<td>.32*</td>
<td>.62**</td>
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<td>--</td>
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</tr>
<tr>
<td>AIR 4 Months</td>
<td>.45**</td>
<td>.75**</td>
<td>.56</td>
<td>.40*</td>
<td>.61**</td>
<td>.71**</td>
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</tr>
<tr>
<td>Depression 12 Months</td>
<td>.46**</td>
<td>.43*</td>
<td>.33^</td>
<td>.26</td>
<td>.38*</td>
<td>.35*</td>
<td>.29^</td>
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</tr>
<tr>
<td>Anxiety 12 Months</td>
<td>.49**</td>
<td>.72**</td>
<td>.50</td>
<td>.20</td>
<td>.66**</td>
<td>.51**</td>
<td>.59**</td>
<td>.70**</td>
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</tr>
<tr>
<td>Stress 12 Months</td>
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<td>.51**</td>
<td>.40*</td>
<td>.18</td>
<td>.49**</td>
<td>.61**</td>
<td>.50**</td>
<td>.74**</td>
<td>.77**</td>
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<tr>
<td>AIR 12 Months</td>
<td>.42^</td>
<td>.68**</td>
<td>.51**</td>
<td>.15</td>
<td>.58**</td>
<td>.51**</td>
<td>.71**</td>
<td>.64**</td>
<td>.78**</td>
<td>.73**</td>
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</tr>
<tr>
<td>Infant 24 hr baseline</td>
<td>.12</td>
<td>-.03</td>
<td>-.12</td>
<td>.11</td>
<td>-.05</td>
<td>-.05</td>
<td>-.04</td>
<td>.38^</td>
<td>.28</td>
<td>.27</td>
<td>.13</td>
</tr>
<tr>
<td>Infant Reactivity b</td>
<td>.12</td>
<td>.21</td>
<td>.19</td>
<td>-.05</td>
<td>.13</td>
<td>.14</td>
<td>.20</td>
<td>.13</td>
<td>.38^</td>
<td>.23</td>
<td>.16</td>
</tr>
<tr>
<td>Infant Recovery b</td>
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<td>-.68**</td>
<td>-.68**</td>
<td>.14</td>
<td>-.83**</td>
<td>-.48*</td>
<td>-.43*</td>
<td>-.44*</td>
<td>-.55*</td>
<td>-.39^</td>
<td>-.54^</td>
</tr>
</tbody>
</table>

*<p < .10. *p < .05. **p < .01.

DASS 4 month variables N= 26

Partial correlations controlling for cortisol 24 hours previous.

AIR = Average Impact Rating of Daily Stress
To further test Hypothesis 3 three hierarchical multiple regression models were used to examine whether maternal report of mental health symptoms at 2, 4 and 12 months of age predicted infant baseline cortisol, cortisol reactivity and recovery at 12 months of age. In the first model the dependent variable was infant baseline cortisol and the independent variables were maternal total mental health scores. To determine whether maternal mental health symptoms early in infant development contributed to infant cortisol independently of recent maternal symptoms, total mental health scores from 2 and 4 months after birth were entered at step one and those reported 12 months after birth were entered at step two. The second and third models had infant cortisol reactivity and recovery as dependent variables (respectively). Additionally, infant baseline cortisol was controlled for and entered in step one with total mental health scores 2 and 4 months after birth. Maternal mental health scores 12 months after birth were entered at step two (Table 20).

In partial support of Hypothesis 3, two of the three regression models were significant. Although, overall, Model 1 was not significant, maternal mental health symptoms at 12 months did make a significant contribution to infant baseline cortisol accounting for approximately 46% of its variance \((R = .46, p = .02)\). Model 2 was significant, baseline cortisol made a significant contribution to cortisol reactivity to the Strange Situation stressor indicating that when infant baseline cortisol was higher, cortisol reactivity was lower. However, contrary to the hypotheses maternal mental health symptoms did not predict infant cortisol reactivity. In Model 3, the variables entered at step one accounted for approximately 76% of the variance in cortisol recovery scores with maternal mental health scores 2 months after birth (T4) making a significant individual contribution. Maternal self-report of mental health symptoms, measured by the total score of the DASS, 2 months after birth, was associated with less
infant cortisol recovery from the Strange Situation stressor indicating, on average, that mothers with more mental health symptoms shortly after birth had infants whose cortisol recovery following the stressor was less efficient. Combined, the results of Models 1 and 3 in Table 20 indicated that when mothers’ reported more mental health symptoms, infant baseline level of cortisol was higher and recovery was lower. However, in Model 2 shown in Table 20, higher infant baseline cortisol was the only correlate of infants' cortisol reactivity, indicating that infants with higher baseline cortisol reacted less relative to other infants.

**Hypothesis 4: Attachment Security and Infant Cortisol**

Of the 38 mother-infant dyads, 27 were classified as having a secure attachment relationship and 11 were classified as having an insecure attachment relationship. Of note, no infant was classified as anxious-avoidant. Hence, all 11 classified as insecure were in the subcategory of Anxious-Ambivalent. Attachment disorganisation was not coded in the current study. To test Hypothesis 4 and determine whether infant baseline cortisol, reactivity, and recovery differed for those infants who were securely and insecurely attached, three independent samples $t$-tests were performed with attachment classification as the grouping variable (secure, insecure). The hypothesis was not supported. There were no group differences in infant baseline cortisol ($t = 0.49, df = 28, p = .63$), cortisol reactivity to the Strange Situation stressor ($t = -0.33, df = 27, p = .74$), and cortisol recovery from the stressor ($t = 0.44, df = 31, p = .67$).
Table 20

Results of Regressing Infant Baseline Cortisol Level on Maternal Symptoms of Mental Health in the Postnatal Period (N=28)

<table>
<thead>
<tr>
<th>Variable</th>
<th>B</th>
<th>SE B</th>
<th>β</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1. DV = Infant 24 hour baseline cortisol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Step 1, $R^2 = .00$</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Total Maternal Mental Health Symptoms (T4)</td>
<td>.00</td>
<td>.00</td>
<td>-.02</td>
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<tr>
<td>Total Maternal Mental Health Symptoms (T5)</td>
<td>.00</td>
<td>.01</td>
<td>-.01</td>
</tr>
<tr>
<td>Step 2, $\Delta R^2 = .21$</td>
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</tr>
<tr>
<td>Total Maternal Mental Health Symptoms (T6)</td>
<td>.01</td>
<td>.00</td>
<td>.62*</td>
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</tbody>
</table>

Model 2. DV = Infant Cortisol Reactivity

<table>
<thead>
<tr>
<th>Variable</th>
<th>B</th>
<th>SE B</th>
<th>β</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step 1, $R^2 = .68$</td>
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<tr>
<td>Infant 24 Hour Baseline Cortisol</td>
<td>-.78</td>
<td>.12</td>
<td>-.81**</td>
</tr>
<tr>
<td>Total Maternal Mental Health Symptoms (T4)</td>
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<td>.00</td>
<td>.21</td>
</tr>
<tr>
<td>Total Maternal Mental Health Symptoms (T5)</td>
<td>-.00</td>
<td>.00</td>
<td>-.13</td>
</tr>
<tr>
<td>Step 2, $\Delta R^2 = .01$</td>
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<tr>
<td>Total Maternal Mental Health Symptoms (T6)</td>
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<td>.16</td>
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</table>

Model 3. DV = Infant Cortisol Recovery

<table>
<thead>
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<th>Variable</th>
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<th>SE B</th>
<th>β</th>
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</thead>
<tbody>
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<tr>
<td>Infant 24 Hour Baseline Cortisol</td>
<td>.13</td>
<td>.11</td>
<td>.16</td>
</tr>
<tr>
<td>Total Maternal Mental Health Symptoms (T4)</td>
<td>-.01</td>
<td>.00</td>
<td>-.79*</td>
</tr>
<tr>
<td>Total Maternal Mental Health Symptoms (T5)</td>
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<td>.00</td>
<td>.07</td>
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<tr>
<td>Step 2, $\Delta R^2 = .00$</td>
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<td></td>
</tr>
<tr>
<td>Total Maternal Mental Health Symptoms (T6)</td>
<td>.00</td>
<td>.00</td>
<td>.05</td>
</tr>
</tbody>
</table>

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

DV = Dependent variable.
**Hypotheses 5 and 6: Infant Coping Displays as a Correlate of Infant Cortisol and a Moderator of the Association of Maternal Mental Health with Infant Cortisol**

To test the final two hypotheses, hierarchical multiple regression was used to determine whether infant coping displays during the Strange Situation were associated with infants' cortisol, and whether infant adaptive coping moderated the association between mothers’ mental health symptoms and infant cortisol at 12 months. Infant attachment behaviour scores for proximity seeking, contact maintenance, resistance and avoidance were used as indicators of infants' coping displays. Mothers' mental health symptom level was the ambient level across the postnatal period calculated as the mean of DASS total scores reported when infants were 2, 4 and 12 months of age. As recommended, independent variables were centred prior to forming interaction effects and conducting the regression analysis (Jaccard, Wan, & Turrisi, 1990). Two interaction effects were formed by multiplying centred values of ambient maternal mental health symptoms with each of the two infant adaptive coping variables (proximity seeking and contact maintenance). Although interactions were not anticipated, two additional interaction effects were formed by multiplying centred values of ambient maternal mental health symptoms with each of the two infant maladaptive coping variables (resistance and avoidance) and these were also tested. Interactions between mothers’ mental health symptoms and coping were tested in four regression models with infant cortisol reactivity as the dependent variable (see Table 21) and four regression models with infant cortisol recovery as the dependent variable (see Table 22). In each model, infant baseline cortisol, maternal mental health and a measure of infant coping were entered in step one with the interaction effect entered in step two.

In the four models relating to infant cortisol reactivity (see Table 21), infant coping was not associated with cortisol responses to the Strange Situation stressor after
controlling for infant baseline cortisol and maternal postbirth mental health symptoms. However, the relationship between proximity seeking and cortisol reactivity had a moderate effect size (.30) indicating that more proximity seeking was associated with greater infant cortisol reactivity. However no interaction between infant coping and maternal mental health were significant.

In all four models of infant cortisol recovery (see Table 22), after controlling for infant baseline cortisol and maternal postbirth mental health symptoms, no measure of infant coping was associated with cortisol recovery. Yet, one significant interaction effect was found. Infant coping measured by proximity seeking moderated the relationship between maternal mental health and cortisol recovery. To further examine this interaction, simple slopes analysis was conducted using high (+1 SD) and low (-1 SD) scores on maternal mental health symptoms and infant proximity seeking. The association between mothers' mental health symptoms and infant recovery was more strongly negative among infants with high proximity seeking. When maternal mental health symptoms were low, infants had high and similar levels of cortisol recovery regardless of their proximity seeking level (see Figure 10). Infants' recovery was substantially lower when their mothers' mental health symptoms were high and, among this group, infants with low proximity seeking showed greater recovery than those with high proximity seeking.
Table 21

Results of Regressing Infant Cortisol Reactivity on Maternal Symptoms of Mental Health and the Moderating Effect of Infant Coping at 12 Months (N=28)

<table>
<thead>
<tr>
<th>Variable</th>
<th>B</th>
<th>SE</th>
<th>β</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step 1, $R^2 = .72$</td>
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</tr>
<tr>
<td>Infant 24 Hour Baseline Cortisol</td>
<td>-.70</td>
<td>.11</td>
<td>-.72**</td>
</tr>
<tr>
<td>Ambient Maternal Mental Health Symptoms (MH)</td>
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<td>.00</td>
<td>.05</td>
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<td>Infant Proximity Seeking (PS)</td>
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<td>.01</td>
<td>.30*</td>
</tr>
<tr>
<td>Step 2, $\Delta R^2 = .00$</td>
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</tr>
<tr>
<td>Interaction PS $\times$ MH</td>
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<td>.00</td>
<td>.04</td>
</tr>
<tr>
<td>Step 1, $R^2 = .64$</td>
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<tr>
<td>Infant 24 Hour Baseline Cortisol</td>
<td>-.75</td>
<td>.13</td>
<td>-.77**</td>
</tr>
<tr>
<td>Ambient Maternal Mental Health Symptoms (MH)</td>
<td>.00</td>
<td>.00</td>
<td>.06</td>
</tr>
<tr>
<td>Infant Contact Maintenance (CM)</td>
<td>.00</td>
<td>.01</td>
<td>.10</td>
</tr>
<tr>
<td>Step 2, $\Delta R^2 = .00$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interaction CM $\times$ MH</td>
<td>.00</td>
<td>.01</td>
<td>.09</td>
</tr>
<tr>
<td>Step 1, $R^2 = .64$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infant 24 Hour Baseline Cortisol</td>
<td>-.77</td>
<td>.12</td>
<td>-.79**</td>
</tr>
<tr>
<td>Ambient Maternal Mental Health Symptoms (MH)</td>
<td>.00</td>
<td>.00</td>
<td>.05</td>
</tr>
<tr>
<td>Infant Resistance (R)</td>
<td>-.00</td>
<td>.01</td>
<td>-.08</td>
</tr>
<tr>
<td>Step 2, $\Delta R^2 = .00$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interaction R $\times$ MH</td>
<td>.00</td>
<td>.00</td>
<td>-.06</td>
</tr>
<tr>
<td>Step 1, $R^2 = .64$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infant 24 Hour Baseline Cortisol</td>
<td>-.77</td>
<td>.12</td>
<td>-.79**</td>
</tr>
<tr>
<td>Ambient Maternal Mental Health Symptoms (MH)</td>
<td>.00</td>
<td>.00</td>
<td>.05</td>
</tr>
<tr>
<td>Infant Avoidance (A)</td>
<td>-.01</td>
<td>.01</td>
<td>-.05</td>
</tr>
<tr>
<td>Step 2, $\Delta R^2 = .02$</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Interaction A $\times$ MH</td>
<td>.00</td>
<td>.00</td>
<td>.18</td>
</tr>
</tbody>
</table>

Note. The dependent variable (DV) in every model was infant cortisol reactivity
* $p < .05$. ** $p < .01$. 
Table 22
Results of Regressing Infant Cortisol Recovery on Maternal Symptoms of Mental Health and the Moderating Effect of Infant Coping at 12 Months (N=28)

<table>
<thead>
<tr>
<th>Variable</th>
<th>B</th>
<th>SE B</th>
<th>β</th>
</tr>
</thead>
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<tr>
<td><strong>Step 1, R² = .49</strong></td>
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<tr>
<td>Infant 24 Hour Baseline Cortisol</td>
<td>.25</td>
<td>.12</td>
<td>.33*</td>
</tr>
<tr>
<td>Ambient Maternal Mental Health Symptoms (MH)</td>
<td>-.01</td>
<td>.00</td>
<td>-.64**</td>
</tr>
<tr>
<td>Infant Proximity Seeking (PS)</td>
<td>.01</td>
<td>.01</td>
<td>.24</td>
</tr>
<tr>
<td><strong>Step 2, ΔR² = .09</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Interaction PS × MH</td>
<td>-.00</td>
<td>.00</td>
<td>-.31*</td>
</tr>
<tr>
<td><strong>Step 1, R² = .50</strong></td>
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<td></td>
<td></td>
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<tr>
<td>Infant 24 Hour Baseline Cortisol</td>
<td>.26</td>
<td>.12</td>
<td>.34*</td>
</tr>
<tr>
<td>Ambient Maternal Mental Health Symptoms (MH)</td>
<td>-.01</td>
<td>.00</td>
<td>-.62**</td>
</tr>
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<td>Infant Contact Maintenance (CM)</td>
<td>.01</td>
<td>.01</td>
<td>.21</td>
</tr>
<tr>
<td><strong>Step 2, ΔR² = .05</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interaction CM × MH</td>
<td>.00</td>
<td>.00</td>
<td>.23</td>
</tr>
<tr>
<td><strong>Step 1, R² = .45</strong></td>
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<td></td>
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</tr>
<tr>
<td>Infant 24 Hour Baseline Cortisol</td>
<td>.19</td>
<td>.12</td>
<td>.25</td>
</tr>
<tr>
<td>Ambient Maternal Mental Health Symptoms (MH)</td>
<td>-.01</td>
<td>.00</td>
<td>-.63**</td>
</tr>
<tr>
<td>Infant Resistance (R)</td>
<td>.01</td>
<td>.01</td>
<td>.12</td>
</tr>
<tr>
<td><strong>Step 2, ΔR² = .03</strong></td>
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<td></td>
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<tr>
<td>Interaction R × MH</td>
<td>.00</td>
<td>.00</td>
<td>.34</td>
</tr>
<tr>
<td><strong>Step 1, R² = .45</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infant 24 Hour Baseline Cortisol</td>
<td>.22</td>
<td>.12</td>
<td>.29</td>
</tr>
<tr>
<td>Ambient Maternal Mental Health Symptoms (MH)</td>
<td>-.01</td>
<td>.00</td>
<td>-.63**</td>
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<tr>
<td>Infant Avoidance (A)</td>
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<td>.01</td>
<td>-.11</td>
</tr>
<tr>
<td><strong>Step 2, ΔR² = .01</strong></td>
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<td></td>
</tr>
<tr>
<td>Interaction A × MH</td>
<td>.00</td>
<td>.00</td>
<td>.09</td>
</tr>
</tbody>
</table>

*Note. The dependent variable (DV) in every model was infant cortisol recovery in response to the Strange Situation stressor.
* p <.05. **p <.01.
Figure 10. Infant proximity seeking moderates the relationship between maternal mental health and infant cortisol recovery.
**Discussion**

Associations between maternal cortisol and mental health with infant self-regulatory responses were examined in this longitudinal study. A primary aim of the study was to determine whether maternal prenatal mental health symptoms and stress were associated with a programming effect on infant baseline cortisol and cortisol responsivity to stress. A second aim of the study was to examine maternal mental health and stress in the first postpartum year. Associations between maternal mental health, stress, infant cortisol and attachment were examined to determine the effects of maternal mental health on the concurrent development of the mother-infant attachment relationship and infant self-regulatory patterns. Finally infant emergent coping was examined as a moderator between maternal mental health and infant cortisol.

In support of the programming hypothesis, it was found that mothers with higher cortisol levels during the second trimester of pregnancy, and more fears relating to their appearance throughout pregnancy, had infants with higher baseline levels of cortisol at 12 months of age. However, no associations were found between maternal prenatal stress (psychological or physiological) and infant cortisol *reactivity* to the stressor, once infant baseline level of cortisol was accounted for. As the infant HPA axis is developing during the second trimester of pregnancy the current findings offer support for previous suggestions that the infant HPA axis may be primed for a stressful environment (e.g., Cottrell & Seckl, 2009; Kapoor, Dunn, Kostaki, Andrews, & Matthews, 2006). However, the strength of the correlation is unusually large and inconsistent with previous research (e.g., Tollenaar, Jansen, Riksen-Walraven & De Weerth, 2011). The current findings expand on those reported in Study 1C where mothers’ report of daily stress in the first and third trimesters were associated with higher infant baseline cortisol at 4 months of age. Combined, these findings indicate that maternal psychological and
physiological stress during pregnancy may affect infant development differentially. These data suggest that, whereas the effects of psychological daily stressors during pregnancy have more immediate effects on infant cortisol, possibly due to the stressors being ongoing postbirth; maternal cortisol and anxiety have associations with infants’ baseline cortisol levels. It has been suggested that baseline levels of cortisol may be genetically predetermined, whereas reactivity may more likely be due to postnatal factors (Brennan et al., 2008). The current findings may indicate either a genetic predisposition to higher cortisol or the effects of ongoing maternal anxiety.

However, the findings relating to cortisol responsivity are contrary to the hypotheses that prenatal programming results in more intensified responses to stressors due to structural changes (e.g., Cottrell & Seckl, 2009). Previously Davis and colleagues (2011) found that higher levels of maternal cortisol during the late second and third trimesters were associated with greater infant cortisol responses to the heel stick procedure in neonates, however earlier cortisol exposure was associated with slower rates of behavioural recovery (Davis, Glynn, Waffarn & Sandman, 2011). The authors cited previous research to support their findings (e.g., Noorlander, De Grann, Middeldorp, Van Beers & Visser, 2006; Seckl & Meaney, 2006). Specifically, they noted that the consequence of exposure to cortisol is dependent on the developmental timing during which exposure occurs (Seckl & Meaney, 2006) and further, that mineralocorticoid and glucocorticoid receptors, associated with the negative feedback loop of the HPA axis, had previously been found in the hippocampus from 24 weeks gestation (Noorlander et al., 2006). There is uncertainty regarding when the expression of mineralocorticoid and glucocorticoid receptors in the hippocampus commences. Therefore, it is important to note that trimester two data were collected at about
21 weeks gestation in the current study. Therefore the earlier gestational age of the foetus in the current study may, in part, account for the inconsistency of these findings.

Consistent with previous research, prenatal anxiety was associated with higher infant baseline cortisol (Gutteling, De Weerth, & Buitelaar, 2004; O’Connor et al., 2005) and one aspect of maternal pregnancy-related anxiety, mothers’ fears about their appearance, was consistently associated with higher infant baseline cortisol. Mothers’ who reported more fears about their changing physical appearance during each trimester of pregnancy had infants with higher baseline cortisol at 12 months of age. However, no association of other pregnancy-related fears, about child health or giving birth, were associated with infant cortisol levels. Other studies have found that pregnancy-related anxiety is associated with infant cortisol after birth, as well. For example, Gutteling and colleagues (2004) found that mothers who reported more fear of giving birth to a handicapped child had children with higher baseline levels of cortisol and more cortisol responsivity to stressors at 4 – 6 years of age. Fear of giving birth has also been associated with delayed motor and cognitive development in eight month old infants (Huizink, Robles de Medina, Mulder, Visser & Buitelaar, 2003). Although the findings relating to maternal appearance reported here may relate to constructs not measured in the current study, such as maternal self-esteem, it is noteworthy that the environment may play a role in determining which aspect of pregnancy-related anxiety is the focus of mothers’ attention. In the current cohort, the majority of participants lived close to popular beach holiday destinations in South East Queensland, Australia, where culture places a strong focus on physical aestheticism. Such a focus could explain the current study findings, making fears about appearance particularly relevant in this context.

Compared to relationships between maternal prenatal psychological distress, more consistent relationships were found between mothers’ postnatal mental health
symptoms and infant cortisol. Commencing two months after birth, mothers’ report of daily stress and mental health symptoms were consistently negatively associated with infant cortisol recovery from the Strange Situation stressor. Moreover, these relationships were strongest between maternal report of anxiety and infant cortisol recovery. Mothers’ symptoms of depression were only associated with infant baseline cortisol 12 months after birth. Furthermore, when maternal mental health symptoms were averaged over the entire postnatal period they accounted for almost half of the variance in infant baseline cortisol. In turn, higher baseline levels of cortisol were associated with lower levels of cortisol reactivity to the stressor. This later finding may indicate a ceiling effect of stress on infant cortisol, whereby infants with higher baseline levels of cortisol may be chronically stressed and less reactive to brief stressors. The findings may suggest either a more efficient negative feedback loop within the HPA axis or infant habituation to low level stressors due to the frequency of those experiences. In Study 1C it was reported that more maternal mental health symptoms at two months of age were associated with poorer cortisol recovery from a vaccination stressor at four months of age. However, when combining symptoms across two and four months, the poorest recovery was noted at four months when maternal mental health symptoms were high and infants had initially had better recovery from the vaccination stressor at two months of age. These findings suggest that the developmental timing of stressors in the postnatal environment affect the ongoing development of the infant HPA axis and thereby alter the way the infant responds to stress. These data suggest when mental health symptoms were high, infants had difficulties regulating the stress system once the threat has passed. It is likely that stressors are particularly problematic during the earliest months of life as biobehavioural reorganisation occurs (e.g., Emde, Gaensbauer & Harmon, 1976, as
cited in Gunnar, 2006). Combined with findings from Study 1C the current study adds weight to previous research suggesting that patterns of cortisol responses are set within the first four (Larson, White, Cochran, Donzella & Gunnar, 1998) to six months (Lewis & Ramsay, 1995) of life.

Some researchers have suggested that baseline levels of cortisol may be genetically predetermined while reactivity may more likely be due to postnatal factors (Brennan et al., 2008). A study designed to deconstruct the genetic and environmental aspects of infant cortisol regulation suggested that genetic resilience to stress is likely to be evident in environments high in adversity, rather than more stable environments (Ouellet-Morin et al., 2009) such as those noted in the current cohort. Therefore the current findings relating to baseline cortisol and reactivity may best be explained as the result of chronic, low-grade stressors associated with predominantly subclinical levels of maternal mental health symptoms.

Alternatively, postnatal factors conducive to infant regulation, such as sensitive caregiving, may offer another explanation for our findings regarding lower levels of reactivity as it has been suggested that when caregiving is optimal the HPA axis becomes hyporesponsive (Gunnar & Cheatham, 2003). However, this hypothesis appears less likely in the current cohort when considering the associations between maternal mental health and cortisol recovery.

No differences in infant cortisol level, reactivity or recovery were found when infants classified as securely attached were compared to those who were insecurely attached. This lack of difference may reflect the predominantly subclinical levels of mental health symptoms and good caregiving in this middle class sample. Previously differences have been noted when comparing secure and disorganised infants (Hertsgaard et al., 1995; Spangler & Grossmann, 1993). However disorganisation was
not examined in the current study, a larger sample is needed to examine this possibility in future research.

**Emergent Coping as a Moderator Between Maternal Mental Health and Infant Cortisol**

In Study 1A it was found that coping moderated the relationship between psychological stress and cortisol in mothers. The current study found only weak evidence to suggest that emerging coping in infancy moderated the relationship between maternal mental health symptoms and infant stress responsivity following the Strange Situation stressor. In fact, in the current study, rather than proximity seeking being found to be associated with better recovery from stress, it was found that infants who engaged in more proximity seeking had marginally higher levels of reactivity. Also, it was found that infants had the poorest cortisol recovery from the stressor when they engaged in a high level of proximity seeking behaviour and had mothers who reported a relatively higher level of mental health symptoms. Hence, infants' high proximity seeking, relative to those with low proximity seeking, was detrimental to infant recovery when mothers had a high level of mental health symptoms. It is likely that unmeasured characteristics such as introspection or intrusiveness often associated with mental health symptoms such as depression and anxiety interfere with maternal reflective function resulting in mothers misreading cues, sending fearful cues to their infants or trying to disengage from the infant prior to them being soothed, each of which could interfere with the infants' physiological regulation. Previously, lack of maternal responsivity to infants' cues has been associated with poorer regulation of heart rate and negative affect (Haley & Stansbury, 2003).

**Limitations and Future Directions**
The study had five primary limitations that should be considered in the interpretation of the findings. First, the sample size was not large, despite the high retention rate within the study. Second, there were difficulties with infant saliva collection that further limited the sample size. Third, a few correlations between maternal stress or anxiety and infants' cortisol recovery were unusually high compared to previous research. Replication of the study in a larger, more heterogenous sample is required to clarify these relationships.

Fourth, the sample size limited the ability to statistically control for confounding variables such as maternal mood, time of day, time since last feed, sleep and breastfeeding. All of these measures have been found to be relevant to studies of maternal stress, mental health, cortisol, and infant stress regulation (e.g., Davis, et al., 2007; Egliston, McMahon & Austin, 2007; Scher, Hall, Zaidman-Zait & Weinberg, 2010). Fifth, despite appropriate reliability and validity of the measures used, relying only on maternal report of mental health symptoms was a weakness of this study, as was the use of the EPDS only after birth. However, maternal report of depressive symptoms in particular did not always accord with the researcher’s observations, and as such, inclusion of additional measures such as clinical interview or observational measures such as the Emotional Availability Scales (EA; Biringen, 2008) is recommended for future research. Inclusion of the EA scales would identify maternal characteristics often affected by mental health symptoms (e.g., sensitivity and hostility). Future research may also include maternal cortisol samples prior to pregnancy and in the postnatal year in order to further examine patterns between pregnancy, maternal mental health and cortisol and infant cortisol. Despite these limitations, some important findings were demonstrated in support of a programming effect in the pre- and postnatal environments.
Clinical and Practice Implications

A relationship between maternal mental health symptoms, infant cortisol and emergent coping was demonstrated. At 12 months of age, infant coping did not assist regulation when maternal mental health symptoms were high and this was particularly true when infants engaged in stronger proximity seeking behaviour. It is noteworthy that in this sample of first-time mothers mental health symptoms were predominantly at a subclinical level. Previously, treatments that focus on improving the quality of mother-infant interaction have been shown to negate the effects of PND on infant cognition (Cicchetti, Rogosch & Toth, 2000), and reduce maternal stress (Milgrom, Ericksen, McCarthy and Gemmill, 2005). Expanding Clinical Guidelines to include focussed interventions to address the mother-infant relationship within the context of maternal mental health problems, rather than solely focussing on maternal mental health may improve infant outcomes, and in particular reduce the likelihood of onset of mental health problems associated with regulatory deficits in infancy and childhood.

Conclusions

This longitudinal prospective study demonstrated that prenatal cortisol and anxiety was associated with infant stress at twelve months of age. Ongoing maternal mental health symptoms in the postpartum, particularly those occurring during critical periods of development, were associated with difficulty regulating once a stressor had passed. Future research including maternal cortisol prior to pregnancy may delineate the specifics of relationships between these key variables, and assist in the development of effective clinical prevention and intervention programs.
CHAPTER 7

General Discussion

This four phase research study had several aims. The primary aim was to broaden current understanding of whether a priming effect of prenatal stress affected the developing foetus differentially at varying stages of development and secondly, whether differences in infants self-regulatory development would be observed as an outcome of the prenatal environment. The other aims were to examine prospective and concurrent associations between maternal mental health variables pre-and post-birth to determine risk factors for postnatal depression (PND) and determine the role that maternal mental health symptoms postbirth had on the development of infant HPA axis regulation and the quality of the mother-infant attachment relationship. Individual study aims will be summarised hereafter. The research was grounded in several theories.

Preconception

Barker’s Foetal Programming Hypothesis (1998, 2004) and studies of non-human primates guided the early development of this research. Barker proposed that changes in nutrition and endocrine function in utero resulted in permanent adaptive changes to structural, endocrine and metabolic systems which predisposed the infant to disease in later life. More recently Barker suggested previous research linking low birth weight to higher baseline cortisol later in life (e.g., Phillips et al., 1998) suggested that the hypothalamic-pituitary-adrenal (HPA) axis could be programmed prior to birth (Godfrey & Barker, 2000).

Conception

While Barker’s theory and research focussed on the physical characteristics of maternal wellbeing (i.e., maternal diet and body composition), developmental psychologists expanded his premise to examine the relationship between maternal
psychosocial stress and cortisol in the prenatal period and infant responses to stress (e.g., Diego, Field & Hernandez-Reif, 2005). Empirical studies of human and non-human primates supported this theoretical paradigm, whereby stress during pregnancy was associated with higher baseline levels of cortisol and more cortisol reactivity to stressors in offspring (Clarke, Wittwer, Abbott, & Schneider, 1994; O’Connor et al., 2005; Wadhwa, Dunkel-Schetter, Chicz-DeMet, Porto & Sandman, 1996). Importantly, the studies of non-human primates indicated that timing of stress appeared to affect infant outcomes. Offspring of mothers stressed early in pregnancy, usually measured in the second trimester, compared to mid or late gestation, weighed less at birth and had poorest behavioural and emotional outcomes (Schneider, Roughton, Koehler, & Lubach, 1999).

Expanding this concept to humans, Gunnar contributed seminal reviews (e.g., Gunnar, 2006; Gunnar & Quevedo, 2007) which were paramount in the development of the studies presented here. Additionally, an influential review of the effects of prenatal stress suggested that previous evolutionary benefits of priming effects of prenatal stress (i.e., heightened arousal) may now come at the cost of neurodevelopmental disorders. Talge and colleagues (2007) reported children were “substantially more likely to have emotional or cognitive problems” (p. 245) as well as being vulnerable to depression, anxiety and attention deficit disorders if they were exposed to higher levels of prenatal maternal stress (Talge et al., 2007). In the postpartum, Gunnar (2006) reported that insensitive and intrusive caregiving associated with maternal mental health problems increased the likelihood of “significant activations of stress biology” (p. 119). With this in mind, the studies reported here aimed to increase understanding of whether stress during a particular trimester of pregnancy increased infants’ vulnerability to HPA axis
dysregulation and the role that maternal mental health may play in the ongoing development of the infant stress system.

In the postnatal period the quality of the attachment relationship is an important factor associated with the development of infant coping and cortisol responses (e.g., Gunnar, 2006). Consistent with an attachment theoretical perspective, a study aim was to examine how the infants’ use of mother when distressed affected cortisol responses; and whether patterns of mental health in the postpartum year contributed to increased stress responses. Finally, stress (e.g., Lazarus and Folkman, 1984) and coping (e.g., Skinner & Zimmer-Gembeck, 2007) theories were drawn on to expand understanding of individual differences in mothers' cortisol and infants' stress responses.

**Pregnancy**

Study 1A involved examining associations between maternal cortisol, mothers’ self-reported stress and mental health symptoms, pregnancy-related anxiety and coping during each trimester of pregnancy. The participant group comprised forty nulliparous pregnant women who were referred from medical offices or responded to public advertisements.

**Birth and the Early Postbirth**

Study 1B was partially exploratory in nature and examined potential patterns in prenatal risk factors for postnatal depression (PND) in the same group of mothers. Unconditional growth curve models were used to identify patterns of maternal cortisol, daily stressors, self-report of mental health symptoms, pregnancy-related anxiety and coping throughout pregnancy and latent growth curve modelling was used to determine their predictive ability in the onset of postnatal depression (PND) symptoms. In addition, a model was used to examine the pathways by which prenatal ambient levels of prenatal stress, depression, anxiety and cortisol were associated with PND.
**Parenting**

Study 1C focussed on the maternal correlates of infants’ basal cortisol levels, cortisol reactivity, and cortisol recovery within the context of prenatal variables and maternal mental health symptoms shortly after birth. Study 1D expanded Study 1C to examine associations between the simultaneous organisation of infant cortisol responses and attachment behaviour. Consistent with Ainsworth’s Strange Situation procedure, infant attachment behaviour was used to assess the quality of the mother-infant attachment relationship.

**Summary of Main Findings**

**Study 1A.** When examining associations between maternal cortisol, coping, depression, anxiety and stress during pregnancy, maternal coping was found to play an important moderating role during the second and third trimesters. Specifically, coping moderated the relationship between mothers’ perceptions of daily stress and their cortisol levels during the second and third trimesters and the frequency of stressors and symptoms of anxiety during the third trimester. Those mothers’ with the highest levels of cortisol also reported the most stress and least adaptive coping behaviours. Examination of the relationships between cortisol and these key mental health variables during pregnancy also indicated that maternal cortisol during the second trimester accounted for almost a quarter of symptoms of anxiety during trimester three. Contrary to our hypothesis, maternal cortisol during the first trimester of pregnancy was not related to symptoms of mental health later in pregnancy.

**Study 1B.** Several prenatal correlates of PND were identified in the second study. Women who reported more symptoms of anxiety, more pregnancy-related anxiety and more stress during the second trimester of pregnancy had more PND symptoms two months after birth. Similarly, women who reported more general anxiety
and stress, but not depression, during the third trimester of pregnancy had elevated PND symptoms. However, these results were not found when considering data collected during the first trimester.

Using growth curve modelling, it was found that the pattern of self-reported maternal anxiety across the three trimesters of pregnancy differed between participants, but these patterns were not associated with PND. Instead, it was the initial growth trajectory level (intercept) of symptoms that was associated with more PND symptoms. That is, women who had higher initial levels of anxiety in the growth curve model have more symptoms of PND two months after giving birth.

Because no differences in growth patterns during pregnancy were found (or no significant change across pregnancy was found in other measures), other analyses focused on the ambient levels of mothers' cortisol, stress and mental health during pregnancy. Ambient levels were formed by averaging measures from each trimester of pregnancy. Results indicated that mothers who had higher average cortisol and anxiety relating to their child’s health had more symptoms of PND. Testing the path through which these key prenatal variables became risk factors for PND, prenatal anxiety completely mediated the relationships between ambient levels of cortisol, stress and depression with PND. However, this was true only of general anxiety symptoms and not of pregnancy-related anxiety.

**Study 1C.** In Study 1C, participants completed an assessment when their infants were two and four months of age, and infants' cortisol was measured in response to immunisation when they were two and four months of age. The aim was to determine whether maternal stress and mental health symptoms commencing in trimester 1 of pregnancy were linked to infant stress cortisol reactivity and recovery after birth. Also, mothers' postnatal mental health symptoms were examined. Overall, mothers' higher
cortisol levels and greater perceived stress during pregnancy were associated with higher infant baseline cortisol at two months of age, but lower response and greater recovery from stress at four months. In contrast, mothers who reported more mental health problems two months after birth had infants with slower cortisol recovery at four months. A moderation effect was also found, whereby infants who had mothers with high levels of mental health problems showed less cortisol recovery at 4 months, but this negative association between mothers' mental health problems and infant stress recovery was significantly stronger among infants with high compared to low recovery at 2 months of age.

**Study 1D.** A final assessment was conducted when infants were 12 months of age in order to examine associations between maternal prenatal cortisol, and mental health in the pre- and post-natal periods, and the development of infant self-regulatory responses. Mothers’ prenatal cortisol during the second trimester of pregnancy, and anxiety relating to appearance throughout pregnancy were each associated with higher infant baseline cortisol at twelve months of age. Maternal mental health symptoms measured at two, four and twelve months postbirth were also associated with signs that infants were having more difficulty regulating their stress responses at twelve months of age and these relationships were strongest when examining maternal reports of mental health symptoms two, and four, months postbirth. Additionally, infants who used more proximity seeking to cope during the Strange Situation stressor had poorer cortisol recovery compared to those who used lower levels of proximity seeking when their mothers had more mental health symptoms relative to other mothers.

**Implications**

**What does this mean for the field?** These studies provide support for a range of theories including the foetal programming hypothesis and generalisation of previous
research conducted with non human primates. In their study with rhesus monkeys, Schnieder and colleagues (1999) reported even mild psychological stress during pregnancy had a detrimental impact on infant development with the poorest outcomes recorded in infants whose mothers were stressed earlier in their pregnancy. Examination of developmental timing of the stressors indicates the critical developmental period for the rhesus monkeys was approximately between five and thirteen weeks post conception of a 23 week gestation period (i.e., late first trimester to second trimester). The findings reported here indicated similar trends in humans, with the most pre-post-birth associations found with measures taken in the first and second trimesters of pregnancy. Mothers with higher cortisol during the second trimester had infants with higher baseline cortisol at twelve months of age. Additionally, mothers cortisol in the first trimester of pregnancy was associated with less infant cortisol reactivity to a vaccination stressor at four months of age, whereas self report of stressors at trimester one were associated with greater cortisol recovery from the stressor at two months and higher baseline at four months of age. However, the findings relating to magnitude of cortisol responses to stressors may indicate species specific differences. That is, the infants in the current study had lower magnitude of cortisol reactivity to stressors while Schneider’s primates had elevated magnitude of cortisol reactivity in the prenatally stressed offspring. The differences in direction are most easily explained when we consider that the non-human primates remained separated from their mothers after birth and remained living with offspring from the same stress condition. Their findings may indicate a trend towards co-dysregulation within the rhesus monkey groups, while the current data may reflect adaption to more optimal caregiving.

Theoretically consistent with Barker’s statement that the HPA axis could be programmed (Godfrey & Barker, 2000) the current findings add to a growing number of
recently published studies. Alterations to structural, metabolic and endocrine systems are purported to occur during periods of rapid cell division (Barker, 1998, 2002) when systems are most vulnerable to organising or disorganising effects (Buss et al., 2012). Consistent with this premise, the majority of associations between prenatal factors and ongoing infant development related to the first and second trimesters of pregnancy when foetal systems were undergoing developmental change. Expanding this premise to include the postnatal period, many of these systems continue to mature and reorganise during the first postnatal year and higher levels of mental health symptoms between two and four months postpartum was associated with higher baseline levels of cortisol and difficulty regulating the stress system when activated.

The studies presented in this thesis, commencing during the first trimester, support and expand the recent report of Davis et al. (2011) that maternal psychological and physiological (e.g., cortisol) processes during pregnancy affect infant development differentially. Similarly, Diego’s (2004; 2005) seminal research indicated that the duration of maternal depression throughout the perinatal period had differential effects on newborn physiology and behaviour. Although the direction of these findings relating to infant cortisol responses differs from recent human research (as well as Schneider’s work) these differences may best be explained by the time frame within which data were collected. Whereas, Davis et al. (2011) reported higher maternal cortisol later in pregnancy predicted increased cortisol responses to a stressor soon after birth, a negative relationship between maternal cortisol early in pregnancy and infant cortisol response to a vaccination stressor four months after birth was found in the current study. Brennan and colleagues (2008) suggested that baseline levels of cortisol may be genetically predetermined while reactivity may more likely be due to postnatal factors. Therefore these data, collected four months later than Davis’ study, may reflect the
additive effects of the postnatal environment. However, as higher levels of cortisol have been found in people with depression the role of genetics cannot be overlooked when considering the relationships between maternal stress and cortisol during pregnancy and infant cortisol in the postpartum.

What does this mean for maternal mental health and coping?

The role of coping moderating stress in Study 1A (Chapter 3) is consistent with existing theoretical paradigms (e.g., Lazarus and Folkman, 1994; Skinner & Zimmer-Gembeck, 2007). Importantly, findings suggest stress related increases in cortisol throughout the course of pregnancy are, to an extent, potentially modifiable. This finding is particularly important when one considers that higher ambient levels of cortisol throughout pregnancy and fear for child’s health were associated with more PND symptoms two months after birth (Study 1B, Chapter 4), which in turn was associated with deficits in infants' cortisol regulation at four (Study 1C, Chapter 5) and twelve (Study 1D, Chapter 6) months after birth. The relationships found in Study 1A and 1B support and expand Da Costa and colleagues’ suggestion that targeting hassles early in pregnancy may reduce symptoms of PND (Da Costa, Larouche, Drista & Brender, 2000). The current study also addressed some of the methodological limitations of previous research which examined mothers at varying times during pregnancy. Focussing on patterns of coping and stress throughout pregnancy allowed examination of prenatal stress at discrete times as well as the ambient load throughout pregnancy. Combined, the current data suggested three main ways that coping may affect mother-infant outcomes 1) reducing the effects of psychological distress associated with daily stressors 2) as a result, or independently of this, maintaining lower cortisol levels during pregnancy and 3) minimising postnatal vulnerabilities in infants, associated with prenatal psychological and physiological distress, by reducing the
severity of maternal mental health symptoms after birth. Promisingly, prenatal cognitive-behavioural intervention has already been suggested as a means to modify the postnatal effects of prenatal stress with lower cortisol levels having been shown in the offspring of mother who attended CBT compared to offspring of controls (Urizar & Munoz, 2011).

Several questions related to maternal concerns for appearance were raised in Study 1D. This aspect of pregnancy-related anxiety was consistently associated with higher infant baseline cortisol. That is, mothers’ report of fears regarding their physical appearance during each trimester of pregnancy was positively associated with infant cortisol at 12 months of age. Additionally, Study 1B indicated concerns for appearance early in pregnancy were associated with more symptoms of PND. Several possible reasons for these findings were suggested in the discussion of these chapters (e.g., maternal self esteem, generalised anxiety). However, the frequency with which this variable was associated with poorer outcomes suggests further research is required to increase understanding of the underlying characteristics that this variable taps into. Previous research in this area indicated body image during pregnancy was relatively stable possibly due to shifting of maternal “self standards” (Duncombe, Wertheim, Skouteris, Paxton & Kelly, 2008; p. 513). Moreover, women who reported more body image concerns prior to pregnancy (reported retrospectively) maintained body image concerns throughout pregnancy and reported more depressive symptoms and poorer health behaviours. Clarifying the pathways through which maternal anxiety relating to appearance is associated with poorer infant outcomes would enable early identification and targeted intervention to reduce the impact of these associations.

What does this mean for the mother-child interaction?
The current studies suggest that even sub-clinical levels of maternal mental health problems during pregnancy can effect infant regulation in the short term and contribute to maternal wellbeing during the early postnatal period, which in turn affects infant regulation from stressors, in the longer term. These findings likely indicate a culminative vulnerability towards regulatory difficulties commencing in-utero and gradually becoming more stable during the first year of life. This concept is particularly important when considering the potential implications for both mother and infant in the immediate, and the cumulative effects of the interaction between the two in the longer term. These data suggested that maternal symptoms of mental health at each stage during the postpartum period affected infant developmental outcomes at the subsequent stage. In addition to these developmental vulnerabilities, maternal symptoms of mental health and perception of impact of daily stressors at 12 months had relatively robust negative relationships with infant cortisol recovery from the Strange Situation stressor. The relationship between the quality of the attachment relationship and infant cortisol has been documented previously (e.g., Lewis & Ramsay, 1995). However, the design of this study allowed examination of the simultaneous development of these constructs during the first 12 months of life. It has been suggested that activation of the HPA axis occurs when behavioural coping responses are not available or applicable (Barnett et al., 1999). However these data suggested the higher levels of behavioural coping responses when mothers reported higher levels of mental health symptoms resulted in poorest cortisol recovery from stressors.

Consistent with theories of attachment and child development these findings support the premise that changes exacted on the infant at each stage of development are an outcome of the interaction between the current events and the available pathways shaped by prior events (Belsky & Fearon, 2002). Similarly, maternal responses to the
infant are dependent on their own ability to cope, and respond appropriately, within the context of the meaning derived from the interaction shaped by their own history, including attachment relationships (i.e., reflective functioning; Fonagy & Target, 1997). Importantly, Sroufe (1988) stresses that these relationships between early influences and ongoing life experiences while inextricably linked, are constantly evolving, and as such predispose the infant to both advantages and disadvantages which can serve as a risk or protective factor. More specifically still, researchers have emphasised the nature versus nurture argument when examining this concept of “differential susceptibility” (Belsky & Pleuss, 2009; Belsky, Bakersmans-Kranenburg & van Ijzendoorn, 2007) and extend the argument beyond the attachment relationship. By linking mother and infant stress physiology and attachment the current series of studies has increased understanding of how the quality of the attachment relationship and maternal wellbeing during the first year of life can influence the development of infant stress responses.

**What does this mean for infants?**

Three main aspects of development have been proposed as factors that increase susceptibility to environmental influences both negatively and positively: Namely, genes, temperament and physiological reactivity (Belsky & Pleuss, 2009). Belsky and Pleuss suggest that foetal programming shapes temperament (e.g., Huizink et al., 2002), emotional (e.g., Mohler, Parzer, Brunner, Weibel, & Resch, 2006), and physiological responses (e.g., O’Connor et al., 2005) in the postpartum. Based on this research the authors raised the question “Is there a fetal programming of postnatal programming?” (p. 348). Certainly this question is consistent with those raised earlier from the current studies. Importantly, a central theme of the studies cited by these authors to support their hypothesis, and the literature reviewed in this thesis, is that these outcomes in
infants are associated with maternal mental health symptoms commencing in the prenatal period.

**Treatment Implications**

The current research has highlighted several important implications for treatment. First, the importance of early identification and treatment of sub-clinical levels of prenatal stress has been identified, as has the importance of advocating for inclusion of coping in treatment. Second, Studies 1A (Chapter 3) and 1B (Chapter 4) raised questions regarding mothers’ interpretation of their subjective experience of arousal, associated with activation of the HPA axis both as a result of pregnancy and stress, and its contribution to psychopathology. A hypothesis was offered regarding whether mothers responded, or reacted, to their experience of arousal and attributed their arousal to pregnancy which subsequently resulted in increased pregnancy-related anxiety. Future research including randomised controlled studies with a mindfulness component may test this hypothesis.

Structural equation modelling in Study 1B demonstrated that symptoms of anxiety, measured by the DASS, fully mediated the relationships between ambient levels of prenatal perceived stress from daily hassles, cortisol, depression, on the one hand, and symptoms of postnatal depression on the other hand. In this model, the strongest direct pathway was between cortisol and anxiety and no significant relationship was noted between perception of stress and anxiety, again indicating a relationship between cortisol and anxiety rather than suggesting that anxiety was an outcome of psychological stress. Of note, these relationships were not significant when the measure of general symptoms of anxiety was replaced with pregnancy-related anxiety (i.e., worry). Combined, these findings suggest that physiological components precede cognitive rumination which in turn is associated with increased levels of PND.
Defining the roles that each component contributes in the onset of PND may assist in more effective treatment earlier in pregnancy. As discussed in Study 1A, randomised controlled trials including mindfulness integrated therapy, cognitive behavioural therapy and coping may contribute to this end.

Third, implications for treatment in the postnatal period must also be addressed. As reported in Studies 1C and 1D maternal mental health impacted infant physiological regulation as early as two and four months of age. By 12 months of age, maternal mental health symptoms were significantly negatively associated with infant cortisol recovery from the Strange Situation stressor. A central tenet relating to the development of infant attachment behaviour is that the infant organises his or her behaviour as an outcome of repeated patterns of caregiver behaviour (Sroufe & Waters, 1977). As such, infants’ emergent coping (i.e., attachment behaviour) was organised within the context of the mother-infant relationship, in some cases characterised by maternal mental health symptoms. The current data indicates when mental health symptoms were higher; more intense attempts to cope by the infant were associated with slower cortisol recovery. This may indicate that: more intense efforts were required to alleviate distress; arousal was maintained due to insufficient responses from caregivers, or the intensity of effort required on behalf of the infant; or, this pattern of physiological recovery developed within the context of suboptimal soothing. Importantly, these findings are positive in that, despite the infants’ proximity seeking behaviour being unsuccessful in alleviating stress, the infants still used adaptive, rather than maladaptive, coping behaviour by seeking proximity to another when distressed.

**Methodological Implications**

The multidimensional conceptualisation and operational definition of prenatal stress was a strength of the current thesis. However, the use of multiple measures of
prenatal stress highlighted several unexpected issues that require further discussion. First, the DASS-21 (Lovibond & Lovibond, 1995) has demonstrated sufficient reliability and validity in a broad range of research (e.g., Antony, Bieling, Cox, Enns & Swinson, 1998) and has previously been used to validate measures of pregnancy related distress (e.g., Kornelsen, Stoll & Grzybowski, 2011). However, it has not been validated specifically for use during pregnancy. The reliability of the anxiety subscale changed markedly throughout pregnancy and dropped to an unacceptable level during the third trimester indicating that further research to assess the appropriateness of its use during pregnancy is warranted. As noted in Chapter 3 some questions regarding symptoms of anxiety could also be experienced as a consequence of pregnancy. In contrast the reliability of the PRAQ-R subscales remained stable throughout pregnancy. A recent psychometric evaluation and review of pregnancy-specific measures, including the PRAQ, asserted that global and pregnancy-related anxiety were related, yet different, constructs and pregnancy-specific measures were more consistent in their predictive ability for premature birth (Alderdice, Lynn & Lobel, 2012). However, some of the findings, reported earlier in this thesis, suggest the PRAQ may also tap into other constructs such as introspection, self esteem, and body image. Further research to examine the relationship between these constructs may bring to light additional areas to target in intervention.

In the assessment of symptoms of psychopathology two broad approaches exist within the literature: self-report and clinician-rated measures. The current study relied exclusively on self-report assessment of prenatal stress. Although self-report measures have been established as reliable and valid in the assessment of these constructs, they do not correlate perfectly with clinician ratings. Future research methodologies should consider the use of clinician-rated measures.
Comparison of different self-report measures of similar constructs such as symptoms of depression also yield imperfect correlations. For example, in the current study the relationship of the DASS depression subscale and the EPDS with outcome measures were not consistent. Whereas the EPDS has been widely validated for use with pregnant women no significant relationships were found in the current study. This suggests that quantification of depression symptoms may be significantly influenced by the measurement tool used. In contrast, while some relationships were found using the DASS depression subscale this tool has not been validated for use with pregnant women. Future researchers seeking to measure severity of depression symptoms should carefully select the method used to ensure its suitability.

Health Economic Implications

The current studies provided additional support for the growing body of evidence suggesting that maternal mental health and wellbeing during pregnancy has ongoing health economic implications. Identification and treatment of maternal mental health symptoms and promotion of wellbeing in the prenatal period has the potential to alter health trajectories for mother and infant in both the immediate and long term. Early identification of potentially modifiable maternal characteristics, including those identified here, has the capacity to facilitate smooth transition to parenthood, reduce health service utilisation, improve the quality of the mother-infant relationship and facilitate early return to work if desired. In addition to these immediate effects, seminal reviews indicate prenatal stress is associated with increased risk of developmental disorders and behavioural problems in offspring (e.g., Talge et al., 2007). Additionally, at-risk infants may become increasingly more vulnerable for the development of emotional and behavioural problems as maternal mental health symptoms impact on the ability to parent optimally and soothe the infant when distressed. For the child, these
difficulties in turn can lead to poorer quality of life, poorer relationship functioning, increased lifetime health service utilisation and ultimately increased risk of multigenerational transmission of psychopathology.

**Strengths and Limitations**

Several aspects of this study are noteworthy. First, the prospective, longitudinal design of the study allowed inferences to be drawn from data collected in the first trimester of pregnancy to the end of the first year of life. Moreover, by collecting data prospectively, recall biases, often associated with mental health symptoms and retrospective designs, were minimised. Second, the multidimensional conceptualisation and operational definition of prenatal stress addressed limitations of earlier research. By utilising a variety of self-report measures, physiological data and observation of mother and infant dyads a comprehensive picture of the perinatal period was captured. Finally, the excellent retention rate across nineteen months, and six waves of data collection, maximised information gained from the available sample.

Nevertheless, the study was not without limitations. Difficulties with recruiting participants during the first trimester of pregnancy and time restrictions associated with candidature resulted in a smaller than anticipated sample size. The sample size limited statistical power and analyses performed. Despite the high retention rate, insufficient volume and/or deterioration of infant saliva samples further reduced the available data for analyses. Further, potentially confounding variables such as maternal mood, SES, maternal health, exercise, and time since last sleep were not controlled for statistically which reduces the confidence with which findings relating to cortisol can be interpreted. Moreover, caregiving characteristics have previously been shown to have influence on infant self-regulation (e.g., Crockenberg & Leerkes, 2004) and these were not measured in the current study. Replication and extension of these findings in a larger, more
heterogenous sample is a necessary next step for research. Additionally, recent recommendations for research in this area include multiple measurements of salivary cortisol to establish diurnal patterns (Davis et al., 2011) indicating that confidence may be reduced in the current data, which was collected at single time points. Moreover, Gunnar (2006) suggested measuring changes in cortisol at discrete timepoints and considering small responses as “good” and large responses as “bad” was too simplistic given the complexity of the biology of stress (p. 110). Reliance on maternal adherence to salivary cortisol collection times and procedures relating to sleep and eating, and lack of standardisation of timing for vaccinations and the Strange Situation procedure, also reduce the confidence with which the current findings can be interpreted. Although a 24 hour baseline sample and computation of magnitude scores were used to minimise the effects of these limitations, it is recognised that this may have been insufficient to rule out misleading results.

**Future Directions**

Confidence in the findings reported here would be increased by replication in a larger, more diverse sample. Moreover, future research should adopt recent recommendations for collection of multiple measures of mother-infant cortisol to establish diurnal patterns. Recording maternal sleep, diet and exercise would additionally serve to strengthen the reliability of inferences made from findings. Future researchers should also consider inclusion of clinician-rated interviews to supplement self-report data and control for maternal mood in the first postbirth year in order to test the foetal programming hypothesis more stringently. Inclusion of the EPDS throughout the entire perinatal period in future research would also be beneficial.

Examination of infant outcomes associated with prenatal maternal stress highlighted the importance of further longitudinal research as a means of reducing the
impact of maternal stress on infant development. Specifically, expanding current developmental paradigms of coping to include maternal coping during the critical developmental transition between pregnancy and parenthood may offer information to guide intervention and potentially modify mother and infant outcomes.

Further, the long term associations between maternal fears for appearance and infant outcomes noted in the current thesis requires further examination. In addition to examining maternal attitudes towards their appearance future research should also assess maternal attributions relating to the role their infant may have played in changes to their appearance. Ideally, longitudinal research with first-time mothers would focus on comparison of body image satisfaction and/or body fears prior to, and throughout, pregnancy and into the postbirth year.

**Conclusions**

The series of studies presented here offer support for the foetal programming hypothesis. Relationships between maternal psychological wellbeing commencing in the first trimester and infant physiological and emotional development were reported. Less frequently examined in this area of research, the important protective role of maternal coping during pregnancy was identified, and suggestions were made for further research to include coping to expand understanding of current evidence-based practice for treatment of prenatal stress and PND. Understanding these earliest developmental experiences allows development of the most parsimonious intervention for at-risk mothers and infants and reduces the likelihood of multigenerational transmission of psychopathology.
References


Appendices

Appendix A. Information Sheet

You and Your First Pregnancy: A Griffith University Project about Stress, Coping and Development among First Time Mothers and their Infants

Chief Investigators:  Associate Professor Melanie Zimmer-Gembeck
Telephone: 07 5552 9085
Email: m.zimmer-gembeck@griffith.edu.au

Dr Allison Waters
Telephone: 07 5552 8132
Email: a.waters@griffith.edu.au

Assistant Investigator:  Ms Judith Warner
PhD Candidate
Telephone: 07 5552 9121
Email: j.warner@griffith.edu.au

Mailing Address:  School of Psychology, Griffith University, Parklands Drive, Griffith University, Qld, 4222

Purpose of the project:
● The general purpose of this study is to examine how daily challenges affect you and your infant during and after pregnancy.
● One of the ways we can see the effects of these challenges is through your reporting on your stress and feelings during and after your pregnancy. This will be done by asking you to complete questionnaires.
● A second way we can see these effects is thought a stress hormone, cortisol, found in everyone’s saliva. We will be using a simple technique that only involves you spitting through a straw into a plastic tube.
● A final purpose is to examine cortisol in your infant’s saliva a few months after birth and to observe your relationship with your infant when he/she is 1-year-old.

What we are asking you to do:
● Register with us if you are between 7 and 13 weeks into your first pregnancy.
● Participation involves us being in touch with you 3 times during your pregnancy and 3 times after you have given birth. At each contact, we will ask you to fill out some questionnaires.
● In addition, we will ask you to provide us with saliva samples now and in each of the next two trimesters of your pregnancy.
● After birth, we would like to meet you and your baby at his/her 2-month vaccination and at his/her 4-month vaccination. At these meetings we would like to collect saliva samples from your baby.
● Come to Griffith University around your infant’s first birthday. At this time we will videotape you and your child and ask you to complete some final questionnaire.
The basis by which participants will be selected or screened:

- You don’t have to feel stressed to take part in this study, every new mum we contact is important to our knowledge of prenatal and postnatal stress and infant development.
- It is important for us to meet you and begin assessment during the first trimester of your pregnancy e.g., before you are 13 weeks pregnant.
- Some pre-existing medical problems may mean that you have extra cortisol in your body. If this is the case, the saliva samples you give us will not be a correct indication of your stress levels. So, if you have a history of medical conditions such as skin disorders (e.g., psoriasis and eczema), inflammatory diseases (e.g., asthma, lupus and arthritis), Addison’s disease (hypocortisolism), Cushing’s syndrome (hypercortisolism), lymphoma, or if you are taking medication containing cortisol we thank you for your interest however, unfortunately, you will be unable to take part in this study.

Collection of saliva samples:

- In order to measure your stress hormones we need to collect a small amount of saliva from you. This involves you spitting through a straw into a plastic tube. There is no pain or risk associated with doing this.
- In order to collect saliva from your baby we will show you how to gently place an absorbent tip between your infant’s cheek and gum until it is saturated then store it in an airtight collection bag. Your baby will not be at any risk and there is no pain involved in this procedure.
- When collecting saliva at home we ask you to store the samples in your refrigerator, in airtight bags provided by us, for two days until we meet you and collect it.

Video Taping:

- When your child is 1-year-old, we will contact you to schedule a meeting at Griffith University. At this time, we will ask for your consent again to make a videotape of you and your child.
- This tape will be used to assess how your infant cope in a new situation.
- Only the investigators or members of the research team will view this video.

Expected Benefits:

- While there will be no direct benefits to you by taking part in this study, your participation will help us to understand how daily challenges affect mums and infants during and after pregnancy and the role that cortisol plays in this. As a gesture of our appreciation we will enter you into a draw each time we meet with you and you could win a gift voucher for an infant-related item.

Potential Risks

- The risks associated with your participation in this project are low. However, some people may find supplying saliva samples intrusive. Should this apply to you, please discuss this with us so that we can discuss alternative ways for you to participate.

Your Confidentiality:

- Your participation in this project and all information provided by you will remain confidential.
- The questionnaires completed by you will be allocated a code, and the data and identifiable code will be stored separately in secure filing cabinets in a locked office.
- Any written reports on the findings from this study will describe information from groups of participants and will never identify you or your infant as an individual.
- Saliva samples collected from you will be allocated a code and frozen until analysed. Analysis involves extracting the stress hormone, cortisol, from your saliva. After
analysis saliva samples will be disposed of in accordance with normal laboratory procedure.

- The videotape of you and your child that will be done when your infant is 1-year-old will be allocated a code and stored in a locked filing cabinet. This video will only be viewed by the investigators or members of the research team.

**Voluntary Participation:** Your participation in this project is entirely voluntary and you are free to withdraw from the research at any time without providing an explanation. Your withdrawal will have no repercussions in your relationship, or any future relationship, with Griffith University.

**Further information:** Please feel free to contact the investigator/s at the phone numbers overleaf for further information regarding any aspect of this research project.

**Ethical conduct of this research:** Griffith University conducts research in accordance with the National Statement on Ethical Conduct in Research Involving Humans. Griffith University requires that all participants be informed that if they have any concerns or complaints concerning the manner in which this research is conducted they may be brought to the attention of the researcher or, if an independent person is preferred, contact: the Manager, Research Ethics, Office of Research, Bray Centre, Nathan Campus, Griffith University. Telephone: (07) 3735 5585 or email research-ethics@griffith.edu.au

**Feedback to you:** At the conclusion of this research project a summary of the results will be provided to you should you request it.

**Privacy Statement:** The conduct of this research involves the collection, access and/or use of your identifiable personal information. The information collected is confidential and will not be disclosed to third parties without your consent, except to meet government, legal or other regulatory authority requirements. A de-identified copy of this data may be used for other research purposes. However, your anonymity will at all times be safeguarded. For further information consult the University’s Privacy Plan at www.gru.edu.au/ua/aa/vc/pp or telephone (07) 3875 5585.

**Thank you for considering participation in this project.**
Appendix B. Consent Form for Participants

You and Your First Pregnancy: A Griffith University Project about Stress, Coping and Development among First Time Mothers and their Infants

CONSENT FORM

Chief Investigators: Associate Professor Melanie Zimmer-Gembeck
Telephone: 07 5552 9085
Email: m.zimmer-gembeck@griffith.edu.au

Dr Allison Waters
Telephone: 07 5552 8132
Email: a.waters@griffith.edu.au

Assistant Investigator: Ms Judith Warner
PhD Candidate
Telephone: 07 5552 9121
Email: j.warner@griffith.edu.au

Mailing Address: School of Psychology, Griffith University, Parklands Drive, Griffith University, Qld, 4222

By signing below, I confirm that I have read and understood the information package and in particular have noted that:

- I understand that my involvement in this research will include the completion of some questionnaires, provision of saliva samples on six occasions (3 from me and three from my baby), and one visit to the Psychology Clinic at Griffith University over an 18 month period;
- I have had any questions answered to my satisfaction;
- I understand the risks involved;
- I understand that beyond being entered into draws there will be no direct benefit to me or my baby for our participation in this research;
- I understand that my participation in this research is voluntary;
- I understand that if I have any additional questions I can contact the research team;
- I understand that I am free to withdraw at any time without comment or penalty;
- I understand that I can contact the Manager, Research Ethics, Office of Research, Bray Centre, Nathan Campus, Griffith University. Telephone: (07) 3735 5585 or email research-ethics@griffith.edu.au if I have any concerns about the ethical conduct of the project;
- I agree to participate in the project.

Name __________________________________________________________
Signature _______________________________________________________
Date _____ / _____ / _____
Appendix C. Consent Form for Nurses

You and Your First Pregnancy: A Griffith University Project about Stress, Coping and Development among First Time Mothers and their Infants

By signing below, I confirm that I agree to my contact details being provided to the research team for the purpose of contacting me to arrange for participation in the study of first time mothers, and in particular have noted that:

- I understand that if I participate in this research it will include the completion of some questionnaires, provision of saliva samples on six occasions (3 from me and three from my baby), and one visit to the Psychology Clinic at Griffith University over an 18 month period;
- I understand that beyond being entered into draws there will be no direct benefit to me or my baby for our participation in this research;
- I understand that my participation in this research is voluntary;
- I understand that if I have any additional questions I can contact the research team on 07 5552 9121 or 0406 584 109;
- I understand that I am free to withdraw at any time without comment or penalty;

Name: _______________________________________________________________
Signature: __________________________________________________________________
Telephone: ______________________ Mobile: ________________________________
Email: ___________________________________________________________________
Date _____ / _____ / _____
Appendix D. Advertising Materials

You and your first pregnancy
A study for first time mothers

Prenatal Stress Network
At Griffith University we are working together to find out more about the effects of stress and coping during pregnancy.

Help us to help mums and infants
If you are a first time mum, in the first trimester of your pregnancy, your help is vital to improving lives for mums and children in Australia. Coping with stress and secure attachment are important outcomes we hope to achieve for all children.

To register with us and participate in this research, or for more information, call Judith Warner:
Business Hours: (07) 55 529 121
After Hours: 0406 584 109
Alternatively SMS your name and number to 0406 584 109 or email Judith on j.warner@griffith.edu.au and we will be in touch soon.
Why is this so important?

- Prenatal and early life experiences are important to the development of both your infant and your family.
- Prior to birth, some of these important experiences include exposure to maternal stress, feeling blue and pregnancy related anxiety.
- In the early years after birth these experiences include stress in the family, caregiver health, parenting difficulties, and the early attachment relationship between you and your young child.
- This study will show us how these experiences form foundations for infants’ ability to cope with stress and help with caregiver infant attachment relationships.
- Coping with stress and secure attachment are important outcomes we hope to achieve for all children.

You and your first pregnancy
A study for first time mothers

Prenatal Stress Network
To register with us and participate in this research, or for more information, call Judith Warner:

Business Hours: (07) 55 529 121
After Hours: 0406 584 109

Alternatively SMS your name and number to 0406 584 109 or email Judith on j.warner@griffith.edu.au and we will be in touch soon.

Mailing: School of Psychology, Griffith University, Parklands Drive, Griffith University, Qld 4222
A group of researchers at Griffith University are working together to find out more about the effects of stress and coping during pregnancy.

**We need YOU!**
(You don’t have to be stressed to take part)

If you are a first time mum your help is vital to improving lives for mums and children in Australia. By participating in this study you can help us find out more about prenatal stress, pregnancy, and mother-infant relationships during the first year of life.

When you participate we will enter you into a draw and you could win some gifts for you and your baby.

**We will ask you to:**

- fill out some questionnaires
- provide us with some saliva samples (from you and your baby) so that we can measure cortisol, a stress hormone
- allow us to come to your infant’s 2 and 4 month vaccinations
- come and visit us at Griffith University around the time of your infant’s first birthday

**Your participation will involve:**

- us being in touch with you three times during your pregnancy…
- … and three times after you have given birth

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**At Griffith University we want every child to start life on the right foot**

- the effects of day to day living on your pregnancy
- the effect of stress on you and your baby
- the foods you crave during pregnancy
- how you feel about child birth
- how you cope with being a new mum
- your baby’s temperament
- how your baby learns to cope during his or her first year of life
Appendix E. Instructions for Collection of Saliva

Instructions for Saliva Collection

**Important** During your pregnancy please collect your sample in the late afternoon between 4 and 5 pm or as close to that time as possible.

Approximately 20 minutes before taking a sample please rinse your mouth out with water and refrain from eating or drinking until after you have given a sample.

Step 1: Wash your hands

Step 2: Use the straw to place a saliva sample in the tube provided. Please use the tube marked “Day 1” on the first day and the tube marked “Day 2” on the second day

Step 3: Close the lid tightly

Step 4: Seal the tube in the plastic bag provided

Step 5: Store the sealed bag in your refrigerator until we come to collect it

Thank you! 😊
Appendix F. Instructions for Infant Saliva Collection

Saliva Collection Instructions for Infants Using the Sorbette

To collect enough saliva to analyse we need two Sorbettes each time you collect saliva from your baby. You can repeat the procedure below twice or hold two Sorbettes together at once.

NEVER LET GO OF THE SORBETTE OR LEAVE YOUR BABY UNATTENDED WHILE COLLECTING SALIVA

How

1. Rinse your baby’s mouth with water after feeding and wait 20 minutes before taking a sample.
2. Remove Sorbette from the envelope and close the envelope to protect the remaining Sorbettes from contact with moisture.
3. Place Sorbette under your baby’s tongue or between their gum and cheek. Leave in place for 90 seconds.
4. Place the Sorbette tip down in the yellow cap of the conical tube then place the tube over the purple sticks and snap down securely.
5. Please place in the freezer within two hours.

When:

Four samples in total – 8 Sorbette sticks

1. The day before your baby’s vaccination at approximately the same time as their vaccination. It’s important NOT to take a sample immediately before or after your baby sleeps or after a long car ride.
2. Just before you leave the house to go for your baby’s vaccination.
3. Twenty minutes after your baby’s vaccination.
4. Forty minutes after your baby’s vaccination.
Saliva Collection Instructions for Infants Using the Sorbette

To collect enough saliva to analyse we need two Sorbettes each time you collect saliva from your baby. You can repeat the procedure below twice or hold two Sorbettes together at once.

**NEVER LET GO OF THE SORBETTE OR LEAVE YOUR BABY UNATTENDED WHILE COLLECTING SALIVA**

**How**

6. Rinse your baby’s mouth with water after feeding and wait 20 minutes before taking a sample.
7. Remove Sorbette from the envelope and close the envelope to protect the remaining Sorbettes from contact with moisture.
8. Place Sorbette under your baby’s tongue or between their gum and cheek. Leave in place for 90 seconds.
9. Place the Sorbette tip down in the yellow cap of the conical tube then place the tube over the purple sticks and snap down securely.
10. Please place in the freezer within two hours.

**When:**

Four samples in total – 8 Sorbette sticks

5. The day before your baby’s visit at approximately the same time as their visit. It’s important NOT to take a sample immediately before or after your baby sleeps, or after a long car ride.
6. Just before you leave the house to go for your baby’s visit.
7. Twenty minutes after your baby’s visit.
8. Forty minutes after your baby’s visit.