Maternal prenatal stress and infant postnatal salivary cortisol levels: Does maternal sensitivity moderate the link?

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MPhil

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Abstract

Background: Difficulties regulating biological, behavioural and emotional processes are fundamental to most childhood psychopathology. One possible mechanism for early dysregulation, supported by evidence from the animal literature, is the programming of the foetal hypothalamic-pituitary-adrenal (HPA) axis in utero. Maternal antenatal stress in animals is linked with impaired behavioural and emotional reactivity in offspring, along with alterations in HPA axis function. The effect of maternal rearing behaviours has been studied in rodents and high quality maternal behaviours in the postnatal period moderate the effect of prenatal stress on offspring outcomes. In humans, there is evidence that maternal stress in pregnancy predicts subsequent childhood behavioural difficulties, and emerging evidence that it may alter the function of the infants HPA axis, suggesting that the developing baby could be sensitive to maternal stress hormones in utero and caregiving behaviours in the postnatal period.

Aims: The aims of the present study were three-fold. First, to examine the effect of prenatal stress, as indexed by maternal anxiety and depression symptoms on subsequent infant cortisol levels and reactivity to a social stressor at 6 months of age, taking into account potential confounding variables where appropriate. Second, to examine the potential moderating role of maternal sensitivity in the association between maternal prenatal stress infant cortisol levels. And third, to compare the effect of maternal prenatal stress measured at two gestational time points on infant cortisol outcome.

Method: This was a prospective longitudinal study of a sub-sample of 91 mother-infant dyads, selected from a larger consecutively recruited sample of first time mothers for intensive study within the Wirral Child Health and Development Study based on their varying levels of intimate partner relationship dysfunction. Maternal prenatal stress was measured during the 2nd and 3rd trimesters of pregnancy, indexed by self-report of anxiety and depression symptoms. Infants' salivary cortisol levels were measured before and after a social stressor paradigm, the still-face procedure, at 6 months of age and maternal sensitivity towards her infant was measured in an 8 minute playful interaction during the same laboratory visit.

Results: No main effect of maternal prenatal stress in the 2nd or 3rd trimester of pregnancy on infant HPA axis function was found using multivariate regression analysis controlling for potential confounding variables. There was a significant association between maternal prenatal anxiety during the 2nd trimester of pregnancy in interaction with maternal sensitivity on infant baseline cortisol. Maternal sensitivity moderated the association between high maternal anxiety during the 2nd trimester and high infant baseline cortisol at 6 months of age. This association remained after controlling for potential confounding obstetric outcomes, maternal demographics and concurrent maternal mood.

Conclusions: It appears that the function of infant's HPA axis may be programmed by exposure to maternal stress during pregnancy but that this programming effect is subject to and moderated by the quality of maternal caregiving behaviour in the postnatal period. The importance of the impact of maternal caregiving behaviour on the relationship between maternal stress during pregnancy and infant outcomes is consistent with the animal and emerging human literature on the subject.

Contents

List of tables

List of figures

List of appendices

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1. Introduction

Difficulties regulating biological, behavioural and emotional processes are fundamental to most childhood psychopathology. Understanding the intrinsic and extrinsic factors that lead to emotion dysregulation in infancy is vital in order to identify individuals at risk for childhood behavioural problems. One possible mechanism for early dysregulation, supported by a wealth of evidence from the animal literature, is the programming of the foetal hypothalamic-pituitary-adrenal (HPA) axis in utero. Maternal antenatal stress has been shown to be strongly linked with impaired behavioural and emotional reactivity and other negative neurodevelopmental outcomes in the offspring. These changes may be mediated by the offspring's HPA axis as abnormal hormonal responses to stress have been demonstrated. The effect of maternal rearing behaviours have been studied in rodents and high quality maternal behaviours in the postnatal period have been shown to moderate the effect of prenatal stress on offspring negative outcomes. In humans, there is substantial evidence that maternal stress in pregnancy predicts subsequent childhood behavioural difficulties, suggesting that the developing baby could be sensitive to maternal stress hormones in utero. However the mechanism by which maternal prenatal stress leads to various negative cognitive, behavioural and emotional outcomes in childhood is as yet unknown. There is some limited evidence that prenatal stress is associated with regulation of the HPA axis in infants, but the studies are constrained by conceptual and methodological issues.

The present study will examine the effect of prenatal stress, as indexed by maternal anxiety and depression symptoms on subsequent infant cortisol levels and reactivity to a social stressor at 6 months of age, in prospective longitudinal study of mothers recruited in pregnancy. Analysis will take into account potential confounding variables such as timing of stress in pregnancy, smoking, infant obstetric outcome and maternal postnatal depression or anxiety symptoms where appropriate. The potential moderating role of maternal sensitivity within mother-infant interactions on infant cortisol will also be examined.

In order to set out the rationale for this study a brief discussion of the importance of infant emotional regulation and one of its key components, neuroendocrine function will be undertaken, including a description of the physiology of the HPA axis. This will be followed by a review of the possible consequences of prenatal stress in the animal and human literature.

1.1 Emotion dysregulation

1.1.1 Emotion dysregulation and psychopathology

Although there has been much difficulty in reaching a consensus on a definition of emotion regulation, it is understood by most to refer to the intrinsic and extrinsic processes that allow emotional reactions to be monitored and modified (Thompson, 1994). Some of these biological and behavioural indices of reactivity can be measured in infancy and may be of use in identifying children at risk for developmental psychopathology (Keenan, 2000).

The development of self-regulatory behaviours is widely believed to be one of the most important developmental tasks in childhood because the ability to regulate negative emotions and behaviours associated with them is key to appropriate social functioning. Individuals are born with very different capacities for regulation and reactivity which are the two core dimensions of temperament (Rothbart & Derryberry, 1981). Research indicates that the interplay of these temperamental differences with multiple levels of functioning including behavioural and physiological systems together with parental socialisation leads to the emergence of emotion regulation throughout infancy (Posner & Rothbart, 2000).

As emotion regulation is linked to positive social development (Cole, Michel & Teti, 1994), children who lack emotion regulation capacities are more likely to struggle forming friendships and intimate relationships (Calkins, 1994). This inability to respond to stimuli with well-maintained control leads to unmodulated changes in behavioural and neuroendocrine functioning (Keenan, 2000). Poor emotion regulation demonstrated by greater expression of negative emotions and/or biological indices has

been linked with aggression and the development of childhood conduct disorder (Calkins & Dedmon, 2000; Calkins & Fox, 2002; Hill, Degnan, Calkins & Keane, 2006) and this type of emotionally labile dysregulation has been most widely studied. However, it seems that dysregulation may be a dimensional construct (Keenan, 2000) and that there may be children at risk for psychopathology at the other end of the spectrum who demonstrate restricted emotional responding or behavioural inhibition. This is supported by Calkins and Fox' (2002) findings that over-control of emotions is linked with social withdrawal in childhood.

1.1.2 Neuroendocrine indices of emotion dysregulation

Biological reactivity to stressors is an important aspect of emotion regulation in infants that has generated interest from researchers. Physiological reactivity to stressors can be seen to underlie temperamental and behavioural reactivity and therefore, an adaptive biological response to stress is an important component in the development of well-regulated emotions and behaviour. A dysregulated stress response may render the individual temperamentally vulnerable and increase the risk of developing behavioural and emotional problems. In particular, the HPA axis which coordinates the release of the hormone cortisol in response to stress has been studied, as changes in cortisol levels are an index of the reactivity of the system and the infant's regulatory capabilities.

There is some evidence to show that behavioural states of distress are associated with raised cortisol, at least in the first six months of life (Gunnar, Broderson, Krueger & Rigatuso, 1996; Stansbury & Gunnar, 1994) but there is little stability over time as most infants in adequate care-giving environments develop regulatory capacities that lead to age related decreases in the cortisol response to stress (Ramsay & Lewis, 1994). It also appears that the postnatal environment may affect the development of the HPA axis (Liu, Diorio, Tannenbaum, Caldji, Francis, Freedman, Sharma, Pearson, Plotsky & Meaney, 1997). This highlights the complex relationship between biological and social processes. Biological systems underlie psychological functioning but social and psychological experiences can directly influence the development of theses biological processes (Cicchetti & Cannon, 1999; Liu et al., 1997).

Variations among children in the intensity and frequency of emotional reactions may also depend on the availability of coping strategies that allow self-regulation of emotions and behaviour. Greater physiological adreno-cortical reactions to stress are employed when psychological coping strategies are not available (Spangler & Grossmann, 1993). A number of studies have shown associations between emotion dysregulation indexed by alterations in the HPA axis and concurrent aspects of psychopathology. For example, observed behaviour problems and patterns of cortisol reactivity have been measured concurrently in pre-school children and when the children were grouped according to whether they were under or over-controlled in their social behaviour, there were significant associations with cortisol response to a play session. Children who were predominantly under-controlled displayed lower cortisol levels in response to the play session. Over-controlled social behaviour on the other hand was associated with higher cortisol levels in response to the play session (Granger, Stansbury & Henker, 1994).

Dysregulation of the HPA axis has been associated with both internalizing and externalizing problems in children. Ashman, Dawson, Panagiotides, Yamada and Wilkinson (2002) found that 7-8 year old children who were reported to have clinically significant internalizing symptoms were more likely to show an elevated stress response to a mild laboratory stressor. Cortisol profiles suggestive of a chronically activated HPA axis have also been linked with increased report of depressive symptoms in adolescent girls (Van den Bergh & Van Calster, 2009).

There has been much work over the past two decades implicating HPA axis function with the development of externalizing behaviour problems in childhood. In two recent reviews the authors concluded that the frequently reported associations between low basal cortisol and early onset aggression (McBurnett, Lahey, Rathouz & Loeber, 2000; Van Goozen, Fairchild, Snoek & Harold, 2007) found in the literature do exist but are weaker than previously thought (Alink, van IJzendoorn, Bakermans-Kranenburg, Mesman, Juffer & Koot, 2008; Hawes, Brennan & Dadds, 2009). Alink et al. (2008) discuss the importance of the interaction between environmental influence such as harsh parenting and the function of the HPA axis. Hawes et al. (2009) suggests that the role of the HPA axis in the development of antisocial behaviour may differ across subgroups of children, highlighting children with high

levels of callous-unemotional traits and HPA-axis hypoactivity as a particularly at risk subgroup. Although the research to date has produced some mixed results, further study into the origins of individual differences in stress reactivity and external factors which influence these differences will provide insight into the development of both internalizing and externalizing behavioural disorders.

1.2 The human stress response

The following section describes the human stress response by means of an explanation of both the physiological aspects of stress in humans. Firstly, the function of HPA axis will be summarised and the effect of pregnancy on its action will be explored. Then, the foetal programming hypothesis will be introduced and described in relation to the HPA axis.

Our response to stress is coordinated by two systems, the autonomic nervous system and the HPA axis that work side by side to release the stress hormone cortisol and other mediators that maintain homeostasis. Allostasis is the term given to this maintenance of stability through change which is necessary for survival (McEwen, 2007). However, dysfunctional activation of the HPA system through prolonged and repeated exposure to stressors, can lead to wear and tear to the body and brain. This allostatic load increases the risk of mental and physical health problems and the developing brain may be especially vulnerable to the effects of excess cortisol (Gunnar & Quevedo, 2007). It is hypothesised that increased circulating cortisol levels in the foetus due to maternal antenatal stress may program the growing foetus' HPA axis and adversely affect the infant's cognition, emotional reactivity and behavioural development (O'Donnell, O'Connor & Glover, 2009). The psychobiology of the stress response and its potential role in the foetal programming hypothesis will now be outlined.

1.2.1 The HPA axis

In contrast to the sympathetic nervous system's immediate release of adrenaline that acts on the body to mobilize the fight/flight response, the HPA axis releases glucocorticoids (cortisol in humans, corticosterone in rodents) which take

approximately 20 minutes to reach peak levels and have a more prolonged effect on the body and brain (de Kloet, Rots & Cools, 1996). In response to a stressor the hypothalamus releases corticotrophin-releasing hormone (CRH) which travels through the hypophyseal portal system to the anterior pituitary where it stimulates the release of adrenocorticotropic hormone (ACTH) into the circulation. ACTH acts on the adrenal glands to stimulate the release of cortisol which enters cells throughout the body and brain. Cortisol binds to receptors in cells, enters the nucleus and alters gene transcription. A negative feedback system is in place to shut off the stress response when high circulating levels of glucocorticoids are detected. Activation of glucocorticoid receptors in the hippocampus, hypothalamus and pituitary inhibit CRH production in the hypothalamus (Gunnar & Quevedo, 2007).

There are two types of corticosteroid receptors: glucocorticoid receptors (GR) and mineralocorticoid receptors (MR); (De Kloet, Vreugdenhil, Oitzl & Joels, 1998). At basal levels, cortisol binds readily to MRs in the brain helping to maintain blood pressure, increases neural plasticity, facilitate cerebral glucose availability and make conditions optimal for the sympathetic nervous systems response to stress. When levels are elevated in response to a stressor, GRs become occupied despite their lower affinity for cortisol and the effects of the hormone reverse, become seemingly detrimental. This seems paradoxical but it has been argued that this suppressive response following acute stress is needed to return the body to homeostasis (Sapolsky, Romero & Munck, 2000). In the short term a robust cortisol response is adaptive but when it is excessive and/or prolonged it becomes maladaptive and the associated allostatic load may increase risk of psychopathology.

1.2.2 The HPA axis and pregnancy

In the animal literature, there is clear evidence that prenatal maternal stress leads to long term changes to the structure and function of the infant brain, including regulation of the HPA axis. The hypothesis that these programming effects are at least partly due to the effect of increased glucocorticoids on the developing foetal brain is supported experimentally in rodent and primate studies. In humans the same programming hypothesis is often cited in relation to the findings that prenatal maternal stress is associated with considerably increased risk of behavioural and

emotional problems in childhood (O'Connor, Heron, Golding, Glover & ALSPAC Study, 2003). However, most prospective studies are based on maternal report of stress, usually by symptoms of anxiety or depression, rather than physiological measures and little is known about pregnant women's HPA response to psychological stressors. Pregnancy causes a plethora of hormonal changes and is characterised by an increase in CRH, ATCH and cortisol. Cortisol levels begin to rise at 25 weeks and by late pregnancy reach two times the non-pregnant level. Reactivity to stress also changes during pregnancy. Physiological stress responses (including heart rate variability, blood pressure and cortisol) to external challenge are attenuated when pregnant women are compared with non-pregnant controls. For a review of changes in the stress response during pregnancy see de Weerth and Buitelaar (2005).

To apply the animal model of foetal programming via the HPA axis to humans requires a link between maternal prenatal stress and increased levels of cortisol to be demonstrated. The evidence for this is weak at present (Evans, Myers & Monk, 2008; O'Donnell et al., 2009; Sarkar, Bergman, Fisk & Glover, 2006) but may be confounded by the known dampening of the cortisol response during pregnancy and timing of the sampling during pregnancy. It is clear that more work in this area is needed in order to establish a mechanism for how maternal prenatal stress might program the developing foetal brain. The placenta plays an important role in regulating the foetus' exposures to maternal hormones and recent work has suggested it may be an important factor in understanding the associations between maternal stress as indexed by self-report measures and child outcomes (O'Donnell et al., 2009).

1.2.3 The role of the placenta

By the end of pregnancy, maternal circulating cortisol levels are high but due to the buffering action of the placenta, the foetus is protected from excess exposure. As maternal cortisol crosses the placenta 80-90% is metabolised to an inactive form by a barrier enzyme called placental 11β-hydroxysteroid-dehydrogenase type II (11β-HSD2), leading to foetal cortisol levels being 13 fold lower than maternal levels. Given that foetal levels are so much lower, the 10-20% contribution from mother that does cross the placenta is enough to double foetal cortisol (Gitau, Fisk & Glover, 2001). Rodent studies also suggest that the function of placental 11β-HSD2 can be

altered by maternal factors including prenatal stress (Welberg, Thrivikraman & Plotsky, 2005). These findings have recently been extended to humans. Glover, Bergman, Sarkar and O'Connor (2009) found that maternal anxiety may in fact increase the permeability of the placenta to cortisol through the down regulation of 11β-HSD2.

1.3 The foetal programming hypothesis

It is not a new idea that the quality of the environment the foetus is exposed to will influence the child's development. The relative importance of prenatal as opposed to postnatal factors has been debated over the years (Pauli-Pott, Mertesacker & Beckmann, 2004) and Barker's work in the early 1990s (Barker, 1995) linking low birth weight with increased risk of metabolic and cardiovascular disease in humans brought about renewed interest in the field by drawing attention to the specific effects of the prenatal environment on the developing foetus. His foetal programming hypothesis states that a foetus' adaptation to an adverse early environment results in permanent physiological and metabolic changes that can lead to increased risk of cardiovascular pathology and type 2 diabetes. Barker (1995) mainly focused on nutrition but perturbed hormonal status is also implicated in developmental programming. Maternal stress during pregnancy is known to be associated with preterm delivery and low birth weight (Rondo et al., 2003) as well neurobehavioural development (Van den Bergh, Mulder, Mennes & Glover, 2005). It is known that maternal and foetal cortisol levels are highly correlated, with maternal levels about 13-fold higher (Gitau et al., 2001). This correlation provides evidence for the mechanism of maternal antenatal stress influencing the infant via the developing HPA axis. Alongside this, a wealth of animal research has shown that excess glucocorticoid exposure is a likely mechanism by which poor environmental conditions and maternal psychosocial stress are communicated to the foetus, leading to programming of various aspects of structure and function.

1.4 Animal Studies

1.4.1 Prenatal stress and HPA axis function

Over the past decade, robust evidence linking antenatal stress and infant behavioural and emotional maladjustment that persists into adulthood has been found in studies using rodents and non human primates. Animal studies are able to provide experimental data, controlled for degree and timing of stress in a way that is not possible in human studies due to ethical considerations and can therefore show direct support for the adverse effects of prenatal stress on the developing brain. Weinstock (2001) found that the offspring of prenatally stressed rats displayed more anxious and inhibited behaviour in novel situations, less prosocial behaviour, as well as depressive-like behaviour and atypical sexual behaviour. These changes have been linked with maternal stress hormones by experimentally altering the function of the adrenal glands (Glover & O'Connor, 2002). In studies of rodents and primates, administration of exogenous glucocorticoids or ACTH mimics the effects of prenatal stress and adrenalectomy abolishes the effects on the offspring (Barbazanges, Piazza, Le Moal & Maccari, 1996; Schneider, Roughton, Koehler & Lubach, 1999).

Chronic prenatal stress induced by repeated tail shocks, increases maternal and foetal CRH and corticosterone by more than 30% (Takahashi, Turner & Kalin, 1998) and offspring born to prenatally stressed mothers show permanent changes in the regulation of their own HPA suggesting programming during the prenatal period. However, research findings on the effect of prenatal stress on the regulation of the HPA axis in the offspring have been inconsistent and therefore it is difficult to draw firm conclusions. Factors that may well be confounding the results of this type of research include variation in the timing and nature of the prenatal stressor, the time of day and age at which HPA axis function is tested and the sex of the offspring. Some studies have shown a significant relationship between prenatal stress and HPA axis response to stress although sometimes only in female offspring (Bakker et al., 1998; McCormick, Smythe, Sharma & Meaney, 1995; Weinstock, Matlina, Maor, Rosen & McEwen, 1992) or when the offspring are at a certain age (Henry, Kabbaj, Simon, Le Moal & Maccari, 1994). Weinstock (2005) discusses the variability of the results and concludes that the prenatal stress must be of a sufficient intensity and administered throughout the final week of gestation in rodents to have an effect and that females are more susceptible than males.

Studies have also investigated which aspects of the maternal HPA axis exposed to stress may lead to adverse outcomes in infant HPA axis function. Henry et al. (1994) and Barbazanges et al. (1996) found that exposure to the stress of an open field, produces augmented HPA activity and slower recovery and that this was associated with fewer hippocampal GRs and MRs in prenatally stressed offspring compared to controls. These suggests that the HPA axis dysfunction is likely to be caused by impaired negative feedback (Weinstock, 2005) as individuals with fewer GRs will be less efficient at sensing high levels of circulating glucocorticoids and sending signals to the hypothalamus to inhibit the release of CRH. Experimental manipulation has been used to show that excess corticosterone is at least partly responsible for HPA axis changes in the infant. Hypersecretion of the hormone under stressful conditions was prevented by maternal adrenalectomy and basal level replacement. By doing so, the decrease in hippocampal glucocorticoid receptors and slower recovery post stressor were prevented (Barbazanges et al., 1996). Dysregulation of the HPA axis was reproduced by injecting stress levels of corticosterone into stressed rats that had undergone adrenalectomy.

Schneider, Moore, Roberts and Dejesus (2001) found similar results in non-human primates, demonstrating that offspring of prenatally stressed monkeys displayed more disturbed behaviour including decreased exploration and play and greater HPA axis activation under stressful conditions than controls. Furthermore, injecting ACTH in place of the prenatal stressor reproduced the neurobehavioural disturbances. This seems to be convincing evidence of the role of excess corticosterone/cortisol during pregnancy in the development of HPA axis dysfunction in offspring however the role of other hormones that participate in the stress response such as β-endorphin has not been taken into account. Also, as Weinstock (2005) notes in her review, experiments administering hormones to mimic physiological effects of stress give saline injections to controls. Without a non injected comparison group the effect of the injection, known to be a stressor capable of producing effects in the physiology and behaviour of the offspring, cannot be separated from that of the hormone administered. Despite these issues, the evidence is convincing and has lead to hypotheses that similar links and mechanisms may be occurring in humans (Glover & O'Connor, 2002).

1.4.2 The postnatal environment

The importance of the postnatal environment on behavioural and biological outcomes associated with exposure to prenatal stress has been well documented in rodents and primates (Liu et al., 1997; Maccari et al., 1995; Meaney, 2001; Sanchez, 2006). Cross fostering experiments have shown that the quality of rearing can moderate the programming effect of prenatal stress on the HPA axis. Prenatally stressed offspring reared by dams that are high in licking and grooming behaviour are less anxious and better able to regulate HPA axis response to stress as adults, compared to those reared by low licking and grooming dams (Liu et al., 1997). The proposed mechanism is that licking and grooming enhances negative feedback sensitivity by influencing the degree of GR expression in the hippocampus. The more GRs present the quicker the stress response of the HPA system will be terminated and over time this will reduce the allostatic load the individual is exposed to. Licking and grooming increases the number of GRs present in the hippocampus by reducing the methylation of the GR genes which prevents their expression (Weaver, La Plante, Weaver, Parent, Sharma, Diorio, Chapman, Seckl, Szyf & Meaney, 2001). This work raises the question of whether the postnatal environment can moderate any effects of prenatal stress in humans and which aspects of maternal behaviour are important.

The extent to which the postnatal environment influences the brain will depend on its stage of development. External factors are believed to have more of an impact on developing brain structures than mature ones. Rodents undergo considerably more neural and neuroendocrine development postnatally compared to humans (Matthews, 2002) and may therefore be more sensitive to postnatal environmental influences. Non-human primates are born more mature than rats but there are still important physiological differences between species that make direct extrapolation from animal data to human observations unwise. However, due to the obvious difficulties in experimentally manipulating maternal stress in humans and studying the effects on infant behaviour and neuroendocrine function, the data from animal studies provides a valuable insight into possible importance of prenatal maternal stress on subsequent infant mental health.

1.5 Human postnatal maternal behaviour

During the first year of life, an infant's capacity for emotional and physiological regulation is limited and external regulation of arousal by the caregiver is needed. Caregivers who respond sensitively to their infant's signals are thought to be able to provide the required external regulation. Insensitive caregiving behaviours are typically unresponsive and/or intrusive in relation to infant cues, and either way they are non-contingent with the infant's signals and prevent the infant from receiving the appropriate external help with emotion regulation and these insensitive behaviours may themselves be a source of stress. Since deficits in self-regulation across several areas of functioning, are associated with early behaviour problems (Calkins & Fox, 2002), studying the impact of maternal behaviour on the infant's capacity for physiological regulation of the HPA axis may increase understanding of the earliest origins of later adverse socioemotional outcomes.

Bowlby (1969) suggested in his first book on attachment theory that the attachment figure's sensitivity in responding to the infant's signals may be an important contributor to the development of a secure attachment relationship. Ainsworth pioneered the empirical study of maternal behaviour in relation to attachment and concluded that "the most important aspect of maternal behaviour commonly associated with the security-anxiety dimension of infant attachment...emerges as sensitive responsiveness to infant signals and communications" (Ainsworth, Blehar, Waters & Wall, 1978)

There is a large body of literature on the sequelae of attachment security. Various aspects of the parent-infant relationship have been explored as possible risk factors for the development of externalizing behaviour problems and insecure attachment has been found to be predictive of later behaviour problems in children (Shaw, 1998). Preschool children who have insecure attachment relationships with caregivers are less sociable, have greater anger, lower self control and more problematic peer relationships (Carlson & Stroufe, 1995; Thompson, 1999). These children are also more likely to be referred to mental health clinics than their securely attached contemporaries (Greenberg, 1999).

The relationship between maternal sensitivity, defined as the ability to respond appropriately and promptly to the signals of the infant, and attachment security has been studied extensively over the past three decades. In a meta-analysis of 30 relevant studies, de Wolff and van Ijzendoorn (1997) found the combined effect size to be r(1664) = .22 (N=1666), which, according to Cohen (1988) constitutes a medium effect. Interestingly, several aspects of maternal behaviour, such as mutuality and synchrony and positive attitude showed similar effect sizes and a multidimensional approach to the study of sensitive parenting behaviours was suggested.

The Mother-Infant Interaction Global Ratings Scale (Owen, 1992 as cited in McElwain & Booth-LaForce, 2006) created for the National Institute of Child Health and Human Development (NICHD) Early Child Care Research Network (NICHD Early Child Care,Research Network, 1999), was developed to quantify numerous aspects of the mother-infant relationship including, sensitivity, intrusiveness, detachment, positive and negative regard for the infant and dyadic mutuality. Maternal sensitivity has typically been studied as a specific aspect of interaction or in some studies composite scores have been developed to reflect multiple dimensions of interaction. The creation of composite scales may provide ratings more consistent with maternal behaviours observed in home observations (NICHD Early Child Care Research Network, 1999). However, specificity of measurement is reduced and findings may be harder to interpret as the exact contribution of each part of the scale is unknown.

There is evidence in the literature of a specific association between the quality of the maternal-infant interaction and infant HPA axis activity. In a study into the HPA axis response to maternal separation in 9 month old infants, it was found that the presence of a substitute caregiver (the playmate protocol) who was warm and sensitively interactive throughout the separation was associated with significantly lower post-test cortisol levels when compared to a substitute caregiver (the caregiving protocol), who was only responsive to distress and non-interactive throughout the rest of the separation (Gunnar, Larson, Hertsgaard, Harris & Brodersen, 1992). A sensitive interaction was experimentally created by instructing the playmate to settle the infant with toys and continue to play with the baby, supporting his/her positive engagement

with the social and physical environment throughout the separation. This evidence suggests that the infant's stress response is influenced by the sensitivity of the caregiver. Another study investigating maternal sensitivity and psychobiological function in the first year of life found maternal sensitivity to be associated with HPA axis function at 3 and 6 months of age. When compared to infants with at least moderately sensitive mothers, infants with highly insensitive mothers displayed greater cortisol change scores in response to 15 minutes of free play. The novelty of the laboratory playroom together with the strain of playing for 15 minutes at such a young age was deemed to be at least mildly stressful to the infant. The free play produced an increase in cortisol in 60%, 50% and 50% of 3, 6 and 9 month old infants of highly insensitive mothers, respectively, as compared to 32%, 19% and 19%, respectively, for infants with at least moderately sensitive mothers (Spangler, Schieche, Ilg, Maier & Ackermann, 1994). Sensitive maternal behaviour appears to function as a social defence against infant stress but there could be many explanations for the associations found. The increase in cortisol found in infants of insensitive mothers could be a direct effect of the stress of intrusive behaviour or the lack of appropriate regulation of arousal. On the other hand, the animal literature suggests maternal behaviour may have an epigenetic effect on the structure and function of the HPA axis in a way that could render infants of less sensitive mothers more physiologically reactive under conditions of stress.

1.6 Maternal report of prenatal stress in humans

Empirical inquiry into the effects of foetal exposure to maternal stress in the prenatal period may have only recently begun but there is already a considerable body of evidence indicating that maternal experience of stress, often measured by anxiety or depression symptoms, is associated with cognitive, emotional and behavioural problems in the child (Van den Bergh et al., 2005). In the last few years some researchers have begun to explore the possible mechanisms behind these associations, such as the involvement of the infant's HPA axis, however these investigations are very much in their infancy. Before considering the evidence to date, the following section discusses the nature of stress and how the way it is conceptualised influences the measures used to establish its presence during pregnancy.

1.6.1 Conceptualising stress

The term ‘stress’ is so widely used in modern vocabulary that it has taken on multiple meanings. It is used as a noun to refer to either a subjective feeling or the external event causing that state. Also, to “stress” or be “stressed out” are commonly used verbs in the parlance of our time. Within the scientific community definitions of stress are equally varied as so many disciplines have studied different aspects of stress. In general stress is characterised in one of three ways (Mason, 1975) depending on the facet of interest: (1) The internal state of the individual; (2) An external event (or ‘stressor’); or (3) the experience that arises from the transaction between person and environment. Physiologists view stress as a physical state, focusing on the neuroendocrine and other biological changes that occur; whereas psychologists are more likely to put emphasis on an individual’s emotional reaction to an external event.

As developmental research progressed it became clear that a full definition of stress required both these elements along with mention of the transaction between person and environment and how an individual’s appraisal of stress will affect both physiological and psychological reactions to it (Lazarus & Folkman, 1984). Taking this view, stress arises when a person’s perception of potential threat or harm exceeds their resources to cope with it. Lazarus and Folkman (1984) define this threat or stressor as a circumstance that threatens the maintenance of an individual’s physical integrity or psychological wellbeing. They viewed stress as a complex, multivariate process and emphasised the need to measure the inputs into the process (e.g., life events), person variables and belief systems that guide appraisal (e.g., self esteem), mediating processes (e.g., coping strategies and social support) as well as the behavioural and physiological output of the transaction (Lazarus, 1990).

Research into the consequences of stress is dependent on having an appropriate measure of stress which will vary depending on how stress is conceptualised. Isolating and measuring all the processes involved in Lazarus’s (1990) person-environment relationship is not straightforward so stress researchers tend to measure

either stress provoking external conditions or the resulting internal state of the individual.

Holmes and Rahe (1967) initiated the life-events approach to stress measurement by creating the Social Readjustment Rating Scale that gives a score to life-events such as the death of a spouse or divorce based on a normative judgement of the strain the event will put on the individual. Self-report of major life-events and exposure to disasters such as Chernobyl or the World Trade Centre attack have been used in studies examining the effects of antenatal stress but this method has been criticized as it doesn't take into account individual differences in the degree to which an event is stressful or the meaning of that event to the individual. Lazarus and Folkman (1984) argued that an individual's appraisal of a situation is key to whether it is stressful or not and led them to suggest that everyday hassles may be more indicative of stress levels than rare life-events, as there is more emphasis on the person-environment relationship. Daily hassles scales tend to ask the participant to record the number and severity of hassles occurring over a recent specified period which avoids any recall difficulties associated with life-events inventories.

The majority of the literature on the effect of prenatal stress on infant development conceptualised stress as the individual's emotional reaction to external events and therefore used maternal report of anxiety and/or depression symptoms as a measure of the magnitude of stress. Measuring stress as emotional output means that the result of differences in appraisal and coping are intrinsically captured within the index of distress.

1.7 Prenatal stress and its effect on the infant's HPA axis – a review of the literature to date

A comprehensive literature review revealed 14 studies published over the past decade designed to examine the possible effect of a variety of prenatal stressors and/or maternal mood on the HPA axis of the child. Results are mixed and not well replicated, which is not surprising with so few studies using such diverse methodologies (see Table 1 for a summary of the studies). However, despite variation in methodology they all show some association between maternal prenatal stress,

most often indicated by anxiety and/or depression symptoms, and the offspring's HPA axis function.

The prenatal indices of stress studied range from depression diagnoses (Brennan, Pargas, Walker, Green, Newport & Stowe, 2008), depression symptoms (Field, Diego, Dieter, Hernandez-Reif, Schanberg, Kuhn, Yando & Bendell, 2004), PTSD (Yehuda, Engel, Brand, Seckl, Marcus & Berkowitz, 2005), anxiety by clinical diagnostic interview (Grant, McMahon, Austin, Reilly, Leader & Ali, 2009; Kaplan, Evans & Monk, 2008) and self rated anxiety (O'Connor, Ben-Shlomo, Heron, Golding, Adams & Glover, 2005; Van den Bergh, Van Calster, Smits, Van Huffel & Lagae, 2008) to daily hassles (Gutteling, de Weerth & Buitelaar, 2004), perceived stress (Leung, Tasker, Atkinson, Vaillancourt, Schulkin & Schmidt, 2010), stressful life events (Entringer, Kumsta, Hellhammer, Wadhwa & Wust, 2009) or experience of a disaster whilst pregnant (Huizink, Bartels, Rose, Pulkkinen, Eriksson & Kaprio, 2008). The outcome measures are just as varied in terms of their timing and the method used to quantify the infant HPA axis function. Some studies measured salivary cortisol reactivity to a stressor, others diurnal or baseline cortisol levels and one study measured venous cortisol after ACTH stimulation. The age at which HPA axis functioning was tested ranged from a day old to young adults. Sample sizes are generally small with the largest prospective study being based on 119 mother infant dyads (Field et al., 2004) and the smallest including only 24 dyads (Gutteling et al., 2004,. The findings from these studies will now be summarised in terms of their conceptualisation of prenatal stress.

1.7.1 Studies using symptoms of anxiety or depression as an indicator of prenatal stress

Grant et al. (2009) prospectively studied the separate and combined effect of maternal prenatal anxiety disorder, assessed by diagnostic clinical interview between 35 and 39 weeks gestation, and postnatal sensitivity of caregiving on infant's salivary cortisol reactivity to a social stressor paradigm. They found prenatal anxiety and maternal sensitivity to be independent, additive predictors of infant cortisol reactivity. Baron and Kenny (1986) define a moderator as "a variable that affects the strength and/or direction of the relation between an independent or predictor variable and a dependant

or criteria variable". This definition is contrasted with a mediator, which is a variable that "accounts for the relation between the predictor and the criterion" and how and why such effects occur.

In Grant et al.'s (2009) study, infants of prenatally anxious mothers had significantly higher post-test cortisol levels than infants of mothers who did not meet criteria for anxiety disorder during pregnancy, independent of the effects of concurrent postnatal anxiety and depression symptoms. The interaction was due to a significant difference in mean cortisol between 25 and 40 minutes post-stressor, with infants of prenatally anxious mothers showing a small increase in cortisol levels and infants of non-anxious mothers showing a significant decrease in cortisol levels. Interestingly, the infants of prenatally anxious mothers show a decrease in cortisol levels from baseline to 25 minutes post-stressor which is contrary to the expected direction and this study certainly warrants replication.

Maternal sensitivity did not moderate the link between prenatal anxiety and infant cortisol reactivity as predicted. However, there was an interaction between maternal sensitivity and infant cortisol reactivity. Infants of highly sensitive mothers showing little change in cortisol concentration from baseline across the three post-test samples whereas infants of mothers in the low sensitivity group displayed a significant decrease in cortisol levels from baseline to 15 minutes post-stressor and then an increase from 15 to 25 minutes post-stressor. This finding supports the hypothesis that postnatal caregiving influences the function of the infant's HPA axis but the relationship appears to be independent of the mother's prenatal anxiety status. After Bonferroni adjustments for multiple comparisons, the association between prenatal anxiety and infant cortisol was reduced to a trend, $p = <0.1$ possibly due to the small sample size n = 88, with only 17 women meeting DSM-IV diagnostic criteria for an anxiety disorder during pregnancy.

In a similar study, Kaplan et al. (2008) looked at the effect of prenatal maternal psychiatric status in the 2nd trimester of pregnancy on infant baseline cortisol taking into account the effect postnatal caregiving. They found that neither antenatal diagnosis nor maternal sensitivity alone could predict infant baseline cortisol at 4 months of age. However, there was a significant interaction between the two

variables. Infants of women with an antenatal diagnosis of anxiety and/or depression were found to have significantly higher cortisol levels if they received less sensitive parenting, but if the received sensitive parenting cortisol levels were impossible to differentiate from those of infants whose mothers did not have a psychiatric diagnosis. The animal literature shows that rearing style can moderate offspring outcome but this is the first human study investigating the link. However, of 314 pregnant women recruited into Kaplan et al.'s (2008) study, only 33 dyads were included in the analysis. This is a very high attrition rate and leaves a sample with poor statistical power, which may be why no main effect of prenatal psychiatric status on infant cortisol was found. The results of this study provide evidence that in humans as well as animals, epigenetic programming of foetal stress physiology can be countermanded by the quality of the postnatal environment.

Brennan et al. (2008) undertook a comprehensive study into the effects of lifetime maternal prenatal anxiety and/or depression diagnosis, assessed retrospectively by structured clinical interview, on cortisol reactivity to stressors in 6-month-old infants. They found comorbid anxiety and depression predicted infant cortisol reactivity and prenatal depression was also associated with increased baseline and mean cortisol concentrations, replicating previous findings (Diego, Field, Hernandez-Reif, Cullen, Schanberg & Kuhn, 2004). However, the retrospective design and cross-sectional nature of this study limits the strength of these findings.

In a prospective study O'Connor et al., (2005) examined the long-term associations between prenatal anxiety and later HPA axis function. Diurnal cortisol profiles were measured in 74 10-year-old children whose prenatal exposure to maternal anxiety and depression was measured by self-report of symptom at 18 and 32 weeks of pregnancy. Prenatal anxiety was positively associated with children's subsequent awakening and afternoon cortisol levels. Van den Bergh et al. (2008) also found that exposure to maternal prenatal anxiety was associated with a high, flattened day-time salivary cortisol profile in 14-15 year olds of both sexes, which has been previously found to be a sign of a chronically activated or hyperactive HPA axis (McEwen, 2007). Both studies looked at the effects of prenatal exposure to maternal anxiety in later childhood, providing evidence in humans of the longer term impact on the HPA axis. Interestingly Van den Bergh at al.'s (2008) study was the only one to look for an

association between HPA axis dysregulation and any psychiatric symptoms in the offspring. It was found that in female adolescents only, the flattened diurnal cortisol profile was associated with depressive symptoms.

Field et al. (2004) and Diego et al. (2004) studied the effect of maternal prenatal depression on newborns' urinary cortisol levels and both found foetal exposure to maternal depression, assessed by self-report of symptoms during the 2nd trimester of pregnancy, was linked with raised cortisol in newborns. Field et al. (2004) also found maternal depression during pregnancy was associated with low birth weight and prematurity and that the effect of maternal depression on prematurity was mediated by maternal cortisol levels.

1.7.2 Studies using negative life-events as an indicator of prenatal stress

In a retrospective study comparing the HPA response to stress in (n = 31) young adults of Western European decent, whose mothers experiences major negative life events during pregnancy to a non exposed comparison group (n = 30); Entringer et al. (2009) found that prenatal stress predicted a greater increase in cortisol in response to a social stress paradigm, despite lower pre-test levels. Individuals who had been exposed to prenatal stress also showed lower cortisol levels following ACTH challenge. There was no difference between the groups in diurnal cortisol levels.

Huizink et al. (2008) found that exposure to the Chernobyl disaster during pregnancy was associated with salivary cortisol levels in the adolescent offspring of both sexes and that the effect was strongest if they were exposed from the 2nd trimester onwards. A single measure of salivary cortisol, taken before a structured interview was analysed after being adjusted for time of day, to take into account circadian rhythms. Information on food intake was recorded but did not significantly contribute to variation in cortisol levels. A similar study (Yehuda et al., 2005) looked at how the stress of being exposed to the World Trade Centre attacks whilst pregnant impacted the developing baby' HPA axis, focusing specifically on the effect of maternal post traumatic stress disorder. Interestingly mothers and infants of mothers who developed PTSD had lower cortisol levels than those who were exposed but did not develop PTSD. There was no comparison made to a non exposed group. This is association

between PTSD and lower cortisol is consistent with finding in previous work (Yehuda, Teicher, Trestman, Levengood & Siever, 1996).

These results suggest that different types of stress may have very different physiological effects and effects on the developing HPA axis and different mechanisms may be involved which highlights the importance of the type of stressor or stress response assessed and psychopathology examined in the research. Cortisol has many biological functions and interpreting disturbances in the axis should be done with caution as there is no set point to define hyper/hyposecretion compared to normal levels. Not only has elevated cortisol been linked to anxiety and depression (Goodyer, Herbert, Tamplin & Altham, 2000; Greaves-Lord et al., 2007; Van den Bergh, Van Calster, Pinna Puissant & Van Huffel, 2008b) cortisol hyposecretion has also been linked with psychiatric disturbances such as PTSD and conduct disorder (Hawes et al., 2009). Future studies need to determine whether there is an optimum level of cortisol secretion and more work needs to be done to ascertain why HPA axis dysfunction, be it high or low cortisol, is associated with different psychological disturbances.

1.7.3 Studies using other measures of prenatal stress

Gutteling et al. (2004) prospectively measured prenatal stress (at 15-17 weeks gestation) in terms of maternal daily hassles and pregnancy specific anxieties, such as the fear of bearing a handicapped child to look for any association with HPA axis function in the 4-5 year old children of these women. More maternal daily hassles and greater fear of bearing a handicapped child were associated with higher overall concentrations of salivary cortisol in children across 5 samples taken on the day they received a vaccination. Results must be interpreted with caution due to limitations of the study. These include the absence of any measure of concurrent maternal stress levels of the mother or her parenting skills. Gutteling et al. (2004) also tried to see whether there was any link between prenatal stress and cortisol reactivity, however taking saliva samples pre and post vaccination did not produce a significant increase in cortisol, on average, in either group so no conclusions could be drawn. Methodological issues surrounding cortisol measurement such as how one selects a stressor that is able to produce a significant cortisol response and how one determines

the length of time until maximum levels are reached has been discussed in the literature and shall be reviewed below.

In a separate study, Gutteling, de Weerth and Buitelaar (2005) examined the effects of prenatal stress and cortisol exposure on the function of the HPA system in 29 five year old children in response to the stress of the first day of school. Both prenatal cortisol and pregnancy anxiety were related to the children's cortisol levels, measured by 5 samples taken across the school day, as a reaction to the first school day. Children whose mothers had higher levels of morning cortisol at 24 weeks of pregnancy, and more fear of bearing a handicapped child (one of the items on the pregnancy related anxiety questionnaire) showed higher levels of cortisol during the first day of school compared to cortisol levels recorded during the weekend.

Finally, in a study published this year, Leung et al. (2010) examined the effect of perceived maternal stress during pregnancy on infant stress reactivity at 2 days and 10 months postnatally. Perceived maternal stress during pregnancy was positively associated with neonatal and 10 month postnatal cortisol reactivity scores. They did not mention controlling for baseline cortisol levels, an important consideration when examining physiological reactivity (see section 1.7.5). Of the 84 mothers and newborns who had consented and satisfied all inclusion criteria saliva samples were collected from only 33 neonates and only 26 gave saliva samples at 10 months. Attrition was due to neonates being fed during or within an hour of the scheduled collection time, insufficient salivary volumes for assay and newborns being too distressed to complete the salivary sampling procedure. Data loss was not related to any of the study measures. Even so, results must be interpreted cautiously as they are based on a very small sample size and they need replication with a larger sample. In this study, perceived maternal stress was used as an indicator of the presence of stress during pregnancy. However, the measure was administered in the immediate postpartum period and, therefore, relied on participants' recall ability. This retrospective method is not as reliable as measuring stress during pregnancy and may more closely represent concurrent stress levels. A strength of this study is that cortisol reactivity was measured during the first year of life, rather than during childhood (Gutteling et al., 2004; Gutteling et al., 2005; O'Connor et al., 2005), adolescence (Van den Bergh et al., 2008) or young adulthood (Entringer et al., 2009). Measuring

cortisol reactivity in infancy makes results more reliably relatable to prenatal anxiety as less postnatal environmental confounders will have been of influence. Although it is still important to assess postnatal levels of stress concurrent with infant cortisol outcomes. Despite the limitations, the results of Leung's (2010) study show a significant correlation between perceived maternal stress during pregnancy and infant cortisol reactivity, adding to the growing body of evidence that prenatal stress is associated with greater HPA axis function in response to stress in the offspring.

1.7.4 Associations between timing of prenatal stress and infant HPA function

A small number of studies have explored the associations between timing of antenatal stress and infant HPA axis function. Brennan et al. (2008) compared the effects of lifetime maternal diagnosis of depression with the effects of exposure to prenatal and/or postnatal depression. Greater reactivity in infant cortisol levels were found following exposure to maternal depression in the prenatal and the postnatal period. It is possible that a programming effect on the foetal HPA axis was occurring in the prenatal period and a different mechanism, based on environmental factors, was influencing the HPA axis in the postnatal period. However, if this were the case an interactive or cumulative effect would be expected following exposed to both prenatal and postnatal maternal depression, which was not found. Differences in baseline cortisol levels were associated with lifetime maternal history of depression. There is a possibility this could be due to genetic influences as there was no direct exposure to maternal depression. The retrospective design of this study limits any firm conclusion pertaining to the effect of timing of prenatal stress.

As part of a prospective, longitudinal study into the effect of antenatal maternal anxiety on HPA axis dysregulation in adolescents, Van den Bergh et al. (2008) investigated maternal anxiety symptoms at 12-22, 23-32 and 32-40 weeks of pregnancy in order to establish whether there is a critical period of gestation in which the foetus is especially sensitive to the effects of prenatal anxiety. Analysis of the results found only exposure to prenatal anxiety at 12-22 weeks to be significantly associated with diurnal cortisol profiles in both sexes and depressive symptoms in girls. These findings differ from O'Connor et al.'s (2005) who found that the impact of prenatal anxiety on the infant's HPA axis was strongest when the exposure

occurred at 32 weeks gestation. Further consideration of the effect of timing of prenatal anxiety on infant HPA function is required in future studies.

1.7.5 Methods of analysing cortisol reactivity

Significant correlations between baseline cortisol values and post-test values have been reported. Grant et al. (2009) found Pearson's correlations ranging from r = .27, between baseline and the 40 minute post-test sample, to r = .64, between baseline and the 15 minute post-test sample. This strong correlation indicates that the infants' cortisol response to the still-face procedure was, at least in part, dependant on the level of HPA activation before undergoing the stressor paradigm. To account for this effect, post-test cortisol can be adjusted to correct for initial values using regression analysis. Brennan et al. (2008) reported a negative correlation between baseline and cortisol reactivity values (r = -.47) and, therefore also controlled for the baseline cortisol value in analysis of reactivity. This correlation can be explained in terms of the Law of Initial Value (Wilder, 1958), a well documented negative association between baseline and change scores in physiological responses. The law states that the higher the initial value the smaller is the tendency to rise on stimulation. In other words, with a given intensity of stimulation, the degree of change produced tends to be greater when the initial value of that variable is low. Since individual differences in initial levels of cortisol are inevitable in the study of human physiology and associations have been noted between prenatal stress and infant baseline cortisol levels, Wilder's law merits considerable attention. Although, amongst the studies reported, here focusing on the prediction of child reactivity only Brennan et al. (2008) and Grant et al. (2009) have adopted this approach.

1.8 Limitations and methodological issues

Reviewing these articles has raised some methodological issues, which shall now be discussed. Firstly, some important confounding variables were not always taken into account in the studies reviewed. Birth weight should ideally be examined as a covariate as it is associated with altered HPA function, psychological stress susceptibility, along with numerous behavioural and mental health outcomes in later life (Seckl, 2004). Reduced foetal growth is a marker of a suboptimal environment for

development (Schlotz & Phillips, 2009), and since we know that maternal cortisol levels and prenatal depression and anxiety are each linked to low birth weight and premature delivery (Field et al., 2006; Rondo et al., 2003), it is likely to be important to factor it into the analysis of the effect of prenatal stress on infant stress reactivity. Whether low birth weight is due to prematurity or growth restriction in term babies may be important, as it will reflect different pathophysiological processes. It could also be valuable to examine gestational age and mode of delivery as covariates since stressful delivery has been shown to be associated with cortisol response to stress at 2 months (Miller, Fisk, Modi & Glover, 2005; Taylor, Fisk & Glover, 2000). Brennan et al.'s (2008) study did analyse delivery complications as a potential confounder and found them to be positively related to infant cortisol concentrations. This may be explained by findings that certain types of prenatal anxiety, such as psychosocial stress and fear of childbirth are associated with interventions such as caesarean sections (Johnson & Slade, 2003). Other possible confounders such as maternal use of drugs, alcohol and smoking during pregnancy have not always been commented on in the studies completed to date. Although it is known that prenatal exposure to cigarette smoke is associated with greater cortisol reactivity to several affect eliciting tasks taken from the Laboratory Temperament Assessment Battery (LabTAB) (Goldsmith & Rothbart, 1999) in 7 month old infants (Schuetze, Lopez, Granger & Eiden, 2008).

Some studies (Brennan et al., 2008; Leung et al., 2010) have relied on retrospective reports of maternal anxious or depressive symptomology/stress and their timing during pregnancy; a method limited by recall bias. Prospective measurement of maternal symptoms at different time points during pregnancy is ideally required. It is also important to assess concurrent maternal mood in the postnatal period to test whether it is exposure to stress in utero specifically that may 'program' the developing foetus' HPA axis, rather than the infant's exposure to stress possibly associated with receiving caregiving from a mother with postnatal mood disturbance. Postnatal depression is known to affect the mother's ability to interact sensitively with her infant (Seifer & Dickstein, 2000) and has also been shown to be associated with offspring HPA axis dysfunction (Halligan, Herbert, Goodyer & Murray, 2004) and so studies should ideally control for these effects in order to evaluate the specific relationship of prenatal maternal anxiety and infant cortisol levels. O'Connor et al. (2005) achieved this in his well-designed study that draws from a subset of the large

Avon Longitudinal Study of Parents and Children cohort. These authors found that the association between prenatal anxiety and awakening cortisol remained significant after controlling for multiple measures of maternal anxiety and depression postnatally. This study also had prenatal measures of both anxiety and depression and found it was symptoms of anxiety in and not depression in late pregnancy that predicted morning cortisol levels in the child at age 10. Other studies report different findings. Brennan et al.'s (2008) study focused on the effect of depression and found baseline cortisol in 6 month old infants to be related to peripartum depression whereas cortisol reactivity was only associated with depression when comorbid maternal anxiety was present. "Peripartum depression" was defined any depressive or anxiety episode either during pregnancy or between birth and the day of the Structured Clinical Interview for DSM-IV which was undertaken 6 months postnatally. The relative individual or combined effects of anxiety and depression need to be examined where possible and failure to do so may partly explain mixed results in studies to date. In addition to measurement of important confounding variables, cortisol collection methods are a potential cause of error in all the studies. There are a number of challenges unique to the collection of cortisol in infants as Egliston, McMahon and Austin (2007) describe in a thorough review. The salient points are summarised next.

1.8.1 Cortisol collection

Collection of the sample must be safe, reliable, acceptable to parents and not inductive of stress for the infant. Blood and urine samples generally prove unsatisfactory as they are too invasive and stress provoking. Field et al. (2004) and Diego et al. (2004) measured urinary cortisol in neonates but this method becomes more difficult as infants get older. Most studies measured salivary cortisol using cotton-based absorbent materials. Salivary cortisol is known to accurately reflect biologically active concentrations in the blood (Kirschbaum & Hellhammer, 1989). It is clearly a simple and non-invasive method in adults but the large data loss in infant research, e.g. 61% in Leung et al. (2010) and 62% in Kaplan et al. (2008), suggests it is not as acceptable to infants and may be a source of considerable stress which is unethical and could influence cortisol levels. Assay requires a certain volume of saliva which can be difficult to obtain from infants particularly if they resist the procedure. That some

infants find collection procedure unacceptable also raises the problem of collection bias. The most behaviourally reactive infants, in whom we would expect greater cortisol reactivity, may be more likely to refuse saliva collection leading to type 2 error in the analysis.

Contamination of samples is another source of potential error. Some researchers have used salivary stimulant to increase volumes available for collection but there is mixed opinion about their use as they may alter salivary pH and make some assays more susceptible to interference. Elements in the materials used to absorb saliva from the mouth may potentially react with antibodies used in some assays, therefore researchers are advised to conduct preliminary testing to anticipate possible interference effects. Also, substances present in the infant's mouth, such as foodstuffs or blood due to teething can affect the integrity of the results. Any medications breastfeeding mothers may be taking, not to mention drugs such as caffeine and nicotine can contaminate the sample along with medications the infant is on. Therefore it is important to take a medication history, being particularly vigilant to steroid based medicines and creams. Awareness of these potential contaminators is integral for future study design.

In healthy adults cortisol levels are highest first thing in the morning and fall to lowest levels around midnight. There is considerable debate in the literature around what age this circadian rhythm is established in children. Findings suggest infants show a circadian rhythm by 3 months of age (Santiago, Jorge & Moreira, 1996), others show that patterns of cortisol secretion continue to develop over childhood, specifically until children give up daytime naps (Gunnar & Quevedo, 2007). To reduce inconsistencies due to circadian variation researchers should note the time of arrival in the laboratory, and time of sample collection to allow time of day to be factored into statistical analysis.

When studying cortisol reactivity, diurnal variation becomes less of an issue as it is the difference between pre-stress and post-stress cortisol concentrations that is of interest. However, there is some debate about the time taken to reach peak cortisol secretion post stressor. The general consensus has been that the peak occurs 20 minutes after the stressor (Egliston et al., 2007). However, newer evidence (Ramsay

& Lewis, 2003) has revealed that there may be greater variation between individuals in time taken to reach peak cortisol. Of the studies reviewed that measured cortisol reactivity, Leung et al. (2010) sampled 20 minutes post-stressor, Gutteling et al. (2004) sampled at 15, 20, 25 and 30 minutes post-stressor, Grant et al. (2009) and Brennan et al. (2008) both took multiple samples up to 40 minutes post-stressor. Entringer et al. (2009) sampled serum cortisol 15, 25, 35 and 105 minutes post-stressor. Finally, sleep and feeding patterns can cause variations in cortisol concentrations, de Weerth, Zijl and Buitelaar (2003) found that infants who had eaten a solid meal within the hour before sampling exhibited higher cortisol levels because of a postprandial cortisol surge. Therefore, time of last sleep and feed should be noted by researchers also.

1.8.2 Stressor paradigms

The study of cortisol reactivity and it role in neurobehavioural regulation requires a stressor paradigm that reliably and consistently induces a mean rise in cortisol concentrations across individuals. Achieving this is no easy task for researchers due to ethical constraints and developmental changes that affect the regulation of the HPA axis. From birth to three month of age, a number of different stressor paradigms used in multiple studies have effectively elevated cortisol levels. However, it becomes increasingly difficult to cause a significant rise in cortisol using laboratory tests over the first 2 years of life. Across early childhood, almost no studies have succeeded in finding an appropriate stressor, but as children approach adolescence, provoking a cortisol increase to a mild stressor becomes easier. This has lead researchers to believe that by the end of the first year of life, infants (at least those in supportive care-giving relationships) enter into a period of stress-hyporesponsivness that continues through to adolescence (Gunnar & Quevedo, 2007). The emergence out of this hyporesponsive period may partly explain the increased risk of psychopathology in young adolescence.

A variety of different stressors have been used in studies of cortisol reactivity including physical examination, vaccination, relationship disruption/separation, negative emotion eliciting paradigms. In non-human primates, maternal separation is a powerful stressor capable of activating the HPA axis (Gunnar, Brodersen,

Nachmias, Buss & Rigatuso, 1996), however, separation paradigms used in infant research are very different. In the Strange Situation which is often used as a social stressor paradigm, separation is brief, a maximum of 9 minutes, only 3 of which are spent alone. If the child cries, comfort is provided and if that does not relieve distress quickly the child is reunited with the parent, rendering the paradigm a very mild stressor. This paradigm is stressful enough to consistently produce mean cortisol increases up to 9 months of age but from then on its effectiveness decreases (Gunnar, Tagle & Herrera, 2009) except for insecurely attached children who are also highly fearful (Gunnar et al., 1996; Spangler & Grossmann, 1993; Van IJzendoorn & Vermeer, 2006). This suggests that the HPA response is more strongly activated in situations where the individual lacks psychological strategies to cope with the stress and that most 1 year olds have developed the resources to deal with a brief separation from the attachment figure, hence the Strange Situation becomes less effective at producing a mean increase in cortisol. However, there are reports that prolonged separation continues to be a stressor capable of increasing cortisol concentrations well into early childhood. Van Ijzendoorn and Vermeer (2006) reviewed the literature and found that attending childcare leads to significant increases in cortisol levels from morning to afternoon, especially in children under 36 months. This evidence suggest that laboratory stress paradigms for children beyond 1 year of age may need to involve longer separation periods, perhaps mimicking the conditions of a childcare environment in order to product adequate elevation in cortisol. However, studies typically examine mean differences in cortisol reactivity rather than identifying subgroups of individuals with different profiles. This approach to analysis in studies of the effectiveness of stressor paradigms may mask important individual differences. Gunner et al. (2009) report the proportion of studies in which mean increases of cortisol were elicited as the age of infants under study increases. The authors found 91% of studies of infants less than 3 months old were successful in producing a mean increase in cortisol but in studies examining infants 4-9 months of age this had dropped to 55% and in the 12-24 month age group, 20% of studies reported a mean increase in cortisol post stressor paradigm. Just 9% of studies looking at cortisol reactivity in 2-5 year old children demonstrated a mean increase in cortisol but this increase to 28% in the 6-11 year age group and increased further to 42% in studies examining cortisol reactivity in 12-18 year olds.

1.9 Summary

There is substantial evidence from animal research that supports the hypothesis that prenatal maternal stress can program the offspring's HPA axis to be hyper-responsive in the face of stressors (Barbazanges et al., 1996; Henry et al., 1994; Weinstock et al., 1992; Weinstock, 2005) and that alteration in the HPA axis function appears to be linked with numerous adverse developmental outcomes. Long lasting deficits in learning, attention and memory (Schneider et al., 2001), decreased exploratory behaviours, increased behavioural disturbances (Vallee, Mayo, Dellu, Le Moal, Simon & Maccari, 1997) and even atypical sexual behaviour (Weinstock, 2001) have been demonstrated. Maternal care-giving and the postnatal environment have been shown to moderate the effect of prenatal stress on behavioural outcomes and the offspring's ability to regulate its HPA axis (Liu et al., 1997). The effects reported in the animal literature vary greatly depending on factors such as the nature and timing of the stress exposure and the sex of the offspring, highlighting the complexity of the relationship.

In humans, a robust link between prenatal maternal anxiety and childhood behavioural and emotional problems has been reported (O'Connor et al., 2003; Talge, Neal, Glover & The Early Stress, Translational Research and Prevention Science Network: Fetal and Neonatal Experience on Child and Adolescent Mental Health, 2007; Van den Bergh et al., 2005) and the HPA axis has been suggested as a possible mediator. Research to provide evidence connecting prenatal stress with the programming of the foetal HPA axis is in its infancy and more work is essential to test whether the mechanism that has been demonstrated in animals is applicable to humans. Namely that prenatal stress activates the maternal HPA axis leading to high levels of cortisol in the foetus where it influences brain development and programmes the offspring's own HPA axis to be hyperresponsive or hyporesponsive to stress. The little evidence we have so far suggests that there is an association between prenatal maternal stress and increased cortisol reactivity or basal cortisol in the offspring but the results are highly variable and researchers are faced with numerous methodological challenges in the collection of reliable data. There is great need for future prospective studies of adequate sample size that take important variables such as timing of prenatal stress,

birth weight, concurrent maternal mood and postnatal caregiving into account in order to tease apart the complex nature of the relationship.

1.10 Rationale

We know early emotional reactivity predicts poor behavioural, developmental and social outcomes in later childhood. There is a need to understand the development of biological emotion regulation in infants and what factors may be protective or play an exacerbating role in vulnerable individuals. In order to move towards establishing early interventions to prevent later adverse outcomes, it is vital to understand the earliest contributors to emotion regulation in infancy. There are very few studies of the effect of foetal exposure to maternal stress, usually indexed by symptoms of anxiety or depression or both, on infant HPA axis function in terms of basal cortisol levels and/or cortisol reactivity to a stressor and even fewer that explore the effect of timing of prenatal stress. The current body of literature presents a mixed picture of the relationship between maternal prenatal stress and infant cortisol levels. There is no consensus as to the gestational age at which the developing foetus may be most sensitive to the effects of antenatal stress.

The present study first aims to add to the current literature by examining the effect of maternal prenatal anxiety and depression, measured at two time points during pregnancy, on infant postnatal salivary cortisol levels in a sample over-representative of mothers with high psychosocial risk. In doing so, covariates known to affect the outcome such as maternal smoking (Schuetze et al., 2008) and birth weight (Seckl, 2004) will also be examined. As birth-weight and gestational age are themselves highly correlated it is reasonable to control for one and not the other to avoid limiting the statistical power of the regression analysis by increasing the number of covariates. Salivary cortisol will be measured pre and post the still-face procedure, a social stressor paradigm widely used in developmental research which has been shown to illicit increases in cortisol in 6 month old infants (Haley & Stansbury, 2003).

The literature has demonstrated the importance of sensitive postnatal maternal care in the development of appropriate behavioural and physiological regulation in infants (Calkins & Hill, 2007). However, the impact of sensitive postnatal caregiving on the

HPA axis of infants that have been exposed to prenatal stress has only been investigated in 2 recent studies (Grant et al., 2009; Kaplan et al., 2008). Results suggest that early caregiving may play a moderating role on the association between prenatal maternal stress and infant salivary cortisol levels, but these preliminary findings require replication. The second aim of the current study is therefore to test the moderating role of sensitivity. This will be achieved using a rating of maternal sensitivity obtained from the observation of a separate playful mother-infant interaction at 6 months of age.

1.11 Aims and hypotheses

The present study aims to investigate the association between prenatal maternal stress, measured by levels of self-reported anxiety and depression symptoms at 20 weeks and 32-36 weeks of pregnancy, on infant HPA axis function, indexed by baseline cortisol levels and cortisol reactivity to a social stressor, in healthy 6 month old infants. Based on the available literature, it is expected that infant salivary cortisol levels will differ as a function of maternal prenatal anxiety and/or depression symptoms and that maternal sensitivity will play a moderating role in this association. It also expected that these associations will persist after controlling for relevant covariates including concurrent postnatal maternal anxiety and depression. This leads to the following hypotheses:

Hypothesis 1: High levels of prenatal maternal anxiety or depression will be associated with increased infant basal cortisol levels and increased cortisol reactivity to a social stressor. These effects will be evident after controlling for possible demographic confounds (maternal age, education, deprivation), smoking behaviour in pregnancy, birth weight and timing of cortisol sampling.

Hypothesis 2: Maternal sensitive behaviour towards her infant at 6 months will moderate the association between maternal prenatal anxiety/depression and infant cortisol levels. High levels of maternal sensitivity will buffer the effect of prenatal anxiety/depression on infant cortisol levels.

Table 1 The effect of maternal prenatal stress on the child's HPA axis

Author and year	Title	Study design and Sample size	Measures	Results
Newborns				
Field et al., 2004	Prenatal depression effects on foetus and newborn	Prospective longitudinal N=119	**Mother:** CES-D, STAI, POMS, 1st morning urine cortisol (**2nd trimester)** **Infant:** Brazelton, 1st morning urine cortisol (**newborns)**	Mothers with depressive symptoms prenatally had: higher cortisol levels and this increased cortisol was also seen in their newborns. Poorer performance on Brazelton. More chance of giving birth to a premature low birth weight baby
Diago et al., 2004	Prepartum, postpartum and chronic depression effects on newborns	Prospective longitudinal N=71	**Mother:** CES-D and urine cortisol **(2nd trimester, 2 weeks postpartum)** Neonate: Urine cortisol and Brazelton **(2 weeks of age**)	Newborns of the mothers with prepartum and postpartum depressive symptoms had elevated cortisol
Lundy et al., 1999	Prenatal depression effects on neonates	Retrospective N=63 (36 with depressive symptoms)	**Mother:** CES-D, STAI, POMS, 1st morning urine cortisol **Infant:** Brazelton, 1st morning urine cortisol (**newborns**)	Mothers with depressive symptoms had higher urine cortisol, so did their newborns who also performed less well on NBAS
Infants				
Leung et	Perceived maternal stress	Retrospective	**Mother:** Perceived stress during pregnancy	Perceived maternal stress and

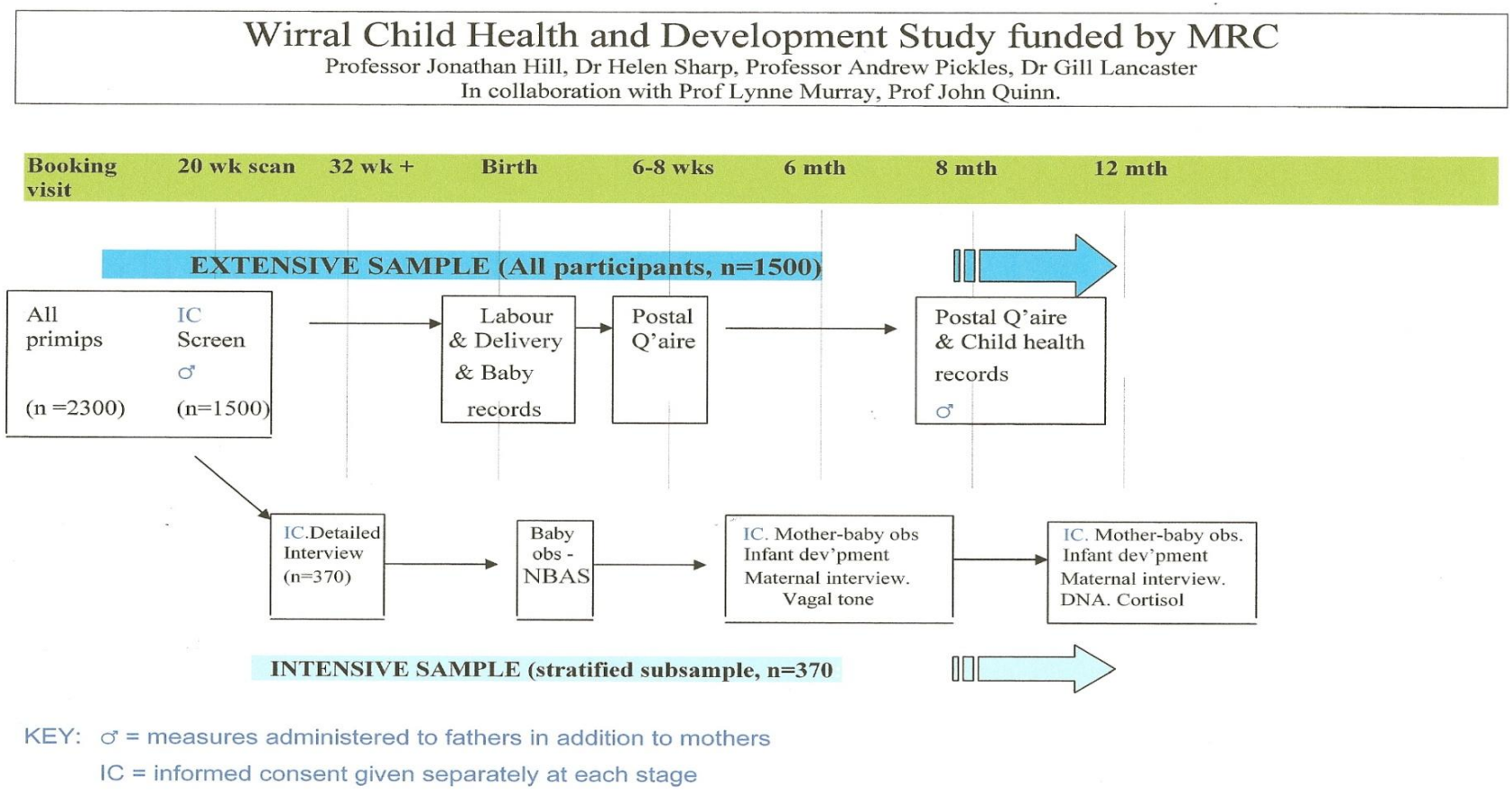
al., 2010	during pregnancy and its relation to infant stress reactivity at 2 days and 10 months of postnatal life	N=33 mother/infants at birth, N=26 by 10 months.	(**assessed 24 hrs from giving birth and at 10 months postnatally**) using Abbreviated Psychosocial Scale **Infants:** Salivary cortisol (24-48hrs) prior to and 20 mins after routine medical exam. Salivary cortisol (**10 months**) baseline and 20 mins post toy removal procedure. Behavioural stress reactivity (10 months): toy removal procedure	infant cortisol reactivity were stable across 1st 10 months of life. Maternal stress during pregnancy predicted infant cortisol reactivity at 2 days and 10 months and behavioural reactivity at 10 months. Neonatal cortisol reactivity predicted behavioural reactivity at 10 months.
Grant et al., 2009	Maternal prenatal anxiety, postnatal caregiving and cortisol response to the still-face procedure	Prospective N=104	**Mother**: Anxiety over past 6/12 (clinical diagnostic interview) at **35-39 weeks gestation** STAI and EPDS postnatally **Infant**: Still-face procedure (**7 months**) Salivary cortisol at baseline and 15, 25 and 40 mins after end of still-face	Prenatal anxiety group showed higher cortisol at 40 min post-procedure. Maternal sensitivity did not moderate the link
Brennan et al., 2008	Maternal depression and infant cortisol: influences of timing, comorbidity and treatment.	Retrospective case control study of **171** mothers and infants	**Mother:** Life time depression and anxiety disorder (SCID) **(recorded 6 months postnatally)** Obstetric health questionnaire (exposure to toxins and medications, delivery complications etc) **Infant:** Salivary coritsol pre (T0) and post (T1) separation stressor. Immediately post arm restraint stressor/noise (T2) and 20 mins later (T3). Post clinical interview (T4) and study exit (T5). (**6 months of age)**	Life time history of maternal depression and anxiety was associated with increased baseline and mean infant cortisol. Peripartum depression (rather than pre-pregnancy) was associated with higher infant cortisol. The association is independent of delivery complication but modulated by prenatal psychotropic treatment.
Kaplan et	Effects of mothers'	Prospective study	**Mother**	Maternal sensitivity modulated

al., 2008	prenatal psychiatric status and postnatal caregiving on infant biobehavioural regulation: Can prenatal programming be modified	of N=47 pregnant women	**2nd Tri**: SCID **3rd Tri**: CES-D & STAI **4/12** assessment: CES-D & STAI, IBQ, maternal sensitivity (10 min free play - EA scales) **Infant**: Heart rate variability, baseline salivary cortisol (all collected between 1-2pm to control for diurnal variations), infant responsiveness (10 min free play) (**4 months of age**)	the effects of psychiatric illness on infant cortisol. Cortisol was low regardless of sensitivity for children of healthy women but higher if infant had insensitive vs. sensitive caregiving when mum had antenatal diagnosis
Yehuda, 2005	Transgenerational effects of posttraumatic stress disorder in babies of mothers exposed to the world trade centre attacks during pregnancy	Prospective, longitudinal epidemiological study of N=38	**Mother:** PTSD Checklist, BDI, morning and evening salivary cortisol **Infant**: Morning and evening salivary cortisol (**9 months)**	Lower cortisol in mothers and babies of mothers who developed PTSD compared to those who didn't. Greatest effect in 3rd trimester exposure
Children (up to age 10)				
O'Connor et al., 2005	Prenatal anxiety predicts individual differences in Cortisol in pre-adolescent children.	Prospective longitudinal cohort study of N=74	**Mother** **18 & 32/40, postnatal – 8 wks, 8/12, 21/12 & 33/12:** Crown-Crisp index of anxiety & EPDS **Child:** Salivary cortisol at awakening, 30 mins after waking, 4pm and 9pm on 3 consecutive days. (**10 years of age)**	Prenatal anxiety was associated with individual differences (elevations) in awakening and afternoon cortisol after accounting for obstetric and sociodemographic risk. Effect of awakening cortisol remained significant after controlling for postnatal anxiety and depression.
Gutteling et al., 2005	Prenatal stress and children's cortisol reaction to the first day	Prospective longitudinal N=29	**Mother:** Questionnaires: Daily hassles, pregnancy related anxiety, perceived stress **(16 weeks gestation)**	Both prenatal cortisol and pregnancy anxiety were related to the children's cortisol levels

	of school.		Salivary cortisol taken at 2hrly intervals between 8am and 8pm in 3 periods of pregnancy **Infant:** Salivary cortisol: 5 samples on a weekend day every 3 h between 0800 and 2000h 4 saliva samples on 1st day of school (repeated 1 week later) (**mean age 5.31 years)**	as a reaction to the first school day. Children whose mothers had higher levels of morning cortisol during pregnancy, and more fear of bearing a handicapped child showed higher levels of cortisol on school days.
Gutteling et al., 2004	Maternal prenatal stress and 4–6 year old children's salivary cortisol concentrations pre- and post-vaccination.	Prospective longitudinal N=24	**Mother**: Daily hassles, pregnancy related anxiety, perceived stress **(at 16 weeks gestation)** Salivary cortisol taken at 2hrly intervals between 8am and 8pm **Infant**: Salivary cortisol baseline and 15, 20, 25, 30 post vaccine. A day curve the weekend before vaccination 3hrly samples between 8am and 8pm **(mean age 4.9yrs)**	Maternal daily hassles and fear of bearing a handicapped child predicted raised cortisol in the child. No cortisol reaction to vaccination so prenatal stress could not be linked to a significantly higher reactivity to the supposed stressor.
Adolescents and young adults				
Entringer et al., 2009	Prenatal exposure to maternal psychosocial stress and HPA axis regulation.	Retrospective case-control N=31 prenatal stress N=30 control	**Mother**: Semi-structured interview about exposure to major negative life events during pregnancy (**administered when offspring are young adults)** Parental bonding inventory (to control for maternal care) **Subjects**: Venous cortisol after ACTH stimulation test Trier Social Stress Test (TSST) Basal diurnal cortisol secretion	Prenatal stress predicted lower cortisol pre-TSST, but a higher increase in response to the test. PS subjects showed lower cortisol following ACTH stimulation. No difference in diurnal levels.

			Corticosteroid binding globulin **Young adults (mean age 25 years**)	
Van den Bergh et al., 2008	Antenatal maternal anxiety is related to HPA-axis dysregulation and self-reported depressive symptoms in adolescence	Prospective N=58	**Mother**: STAI **(12-22, 23-32, 32-40wks)** **Offspring**: Diurnal salivary cortisol (**age 14-15**)	Antenatal anxiety at (12-22wks) was associated with a flattened diurnal cortisol profile. This profile was associated with depressive symptoms in girls.
Huizink et al., 2008	Chernobyl exposure as stressor during pregnancy and hormone levels in adolescent offspring	Retrospective case-control N=121 twins exposed N=157 twins not exposed	**Mother**: Stress not measured directly – pregnant at the time of the Chernobyl disaster **(trimester of exposure noted).** Birth outcome, SES and questionnaires. Self report drinking and smoking during pregnancy **Offspring**: (Baseline) Salivary cortisol and testosterone before and after a structured interview (**age 14)**	Cortisol raised in those exposed from 2nd trimester onwards.

Figure 1 Overview of WCHADS measurement Phases



2. Methods

2.1 Ethical approval

All the data collected in the present study are part of an ongoing, larger study and therefore ethical approval has already been granted by the Cheshire Local Research Ethics Committee and the Research and Development committees for Wirral University Teaching Hospitals NHS Trust, Wirral PCT and Western Cheshire PCT. All ethical considerations regarding the collection and use of maternal psychosocial status, videotaped mother-infant interaction and infant cortisol data in this study have been considered within the bounds of the existing LREC approval (see appendices 3a, b & c).

2.2 Study design

This prospective, longitudinal study is nested within an existing Medical Research Council funded study, The Wirral Child Health and Development Study (WCHADS), which was designed to investigate the earliest origins of childhood conduct disorder. An overview of the measurement phases for the WCHADS is given in Figure 1. The study used a two stage epidemiological sampling strategy to generate an 'extensive' sample and an 'intensive' sample for longitudinal follow up. A sub-sample of women for intensive study was drawn from a consecutive 'extensive' community sample of primiparous women during pregnancy. All women were registered at one maternity unit for their antenatal care, serving the majority of the local population. Women were selected for inclusion in the intensive sample on the basis of level of relationship dysfunction reported at phase 1 screening at around 20 weeks gestation. All women scoring above threshold for relationship dysfunction were eligible for inclusion in the intensive sample. In addition, 14% of women scoring below threshold were randomly selected and were also eligible for inclusion. This strategy was adopted to oversample women likely to be at higher risk for relationship abuse and other co-occurring risks for adverse parenting outcomes such as mental health problems and socioeconomic deprivation associated with the development of behavioural problems in their offspring. The data reported in this thesis is from assessments completed by a subgroup of the WCHADS 'intensive' sample (see section 2.4.4). The two stage study

design was adopted to allow results from the smaller 'intensive' sample to be statistically weighted to the larger consecutive sample (termed the 'extensive' sample); making them generalisable to primiparous women throughout the population once data collection in the whole WCHADS sample was complete. The present study contains data collected during pregnancy from the first two phases of the WCHADS collected at 20 weeks and 32-36 weeks gestation respectively and from Phase 6 of WCHADS when the infants were approximately 6 months old.

2.3 Power calculation for sample size

As there are very few published studies addressing the relationship between antenatal stress and infant cortisol reactivity, there is limited directly comparable literature for estimating effect size. A recently published study (Leung et al., 2010) examined whether perceived maternal stress during pregnancy predicted infant cortisol reactivity at 10 months and found that maternal stress score predicted cortisol reactivity ($B=0.38$; $SE=0.18$; $p< .05$), a medium effect size. The sample in the current study contains a stratum with high psychosocial risk and so a greater or at least equivalent effect size might be expected. Therefore, all power analyses computed were based on the selection of estimated medium effect sizes for the relevant tests.

Power analysis using GPower (Faul, Erdfelder, Lang & Buchner, 2007) for bivariate correlations indicated that a sample size of 55 would have 80% power to detect $r = .36$ with alpha of 0.05. Cohen (1988) defines a medium effect size for correlational analysis in the social sciences as $r = .24$ to .36. A further power analysis using GPower 3 (Faul et al., 2007) for multiple regression was conducted. This analysis indicated a total sample size of 103 participants would be required to detect an effect size of 0.15 (alpha of 0.05) with seven predictors in the model. The power analysis was conducted using the maximum possible number of predictors likely to be included in the regression model. Use of fewer predictors will increase the power of the test. It was not possible to complete an a priori power calculation to detect a possible interaction term.

2.4 Participants

Recruitment took place over a 21-month period at Arrowe Park Hospital on the Wirral. The Wirral is a socio-economically diverse region of North-West England. All primiparous women attending booking appointments (at approximately 12 weeks of gestation) for antenatal care at the maternity unit in the hospital were approached. They were provided with an introductory letter and information sheet about the WCHADS explaining that all first time in the area were being invited to be part of a study aiming to find out more about children's early development. Midwives then asked each woman if she was happy to hear more about the study at her 20 week scan appointment from a research midwife. Written informed consent was subsequently requested at this later appointment as appropriate.

2.4.1 Extensive study: Recruitment and attrition to Phase 1

Figure 2 displays recruitment to the whole WCHADS sample. Of the 1881 women who were eligible for inclusion in the study and who gave permission to be approached, 1268 (68.4%) consented to be part of the study. This high percentage indicates that the consecutive sample of primiparous women is likely to be broadly representative of those registering for antenatal care in that maternity unit.

2.4.2 Intensive study: Recruitment and attrition to Phase 2

Figure 3 displays recruitment to the intensive study. Of the 554 women allocated to intensive study, based on their screening scores for intimate relationship functioning, 341 (61.6%) consented to intensive study and gave Phase 2 data. These women were compared to those who declined participation in the intensive study and were found to report similar screening scores on the measure of intimate relationship functioning at original recruitment. The non participant group was significantly younger and more deprived.

2.4.3 Intensive study: Attrition to Phase 6

Retention in the intensive sample was high. Of the 341 women who consented and gave data at Phase 2, 299 (87.7%) were retained to Phase 6 and 279 provided data. Figure 4 summarises the numbers and reasons for attrition.

Figure 2 Extensive study: Recruitment and attrition to Phase 1

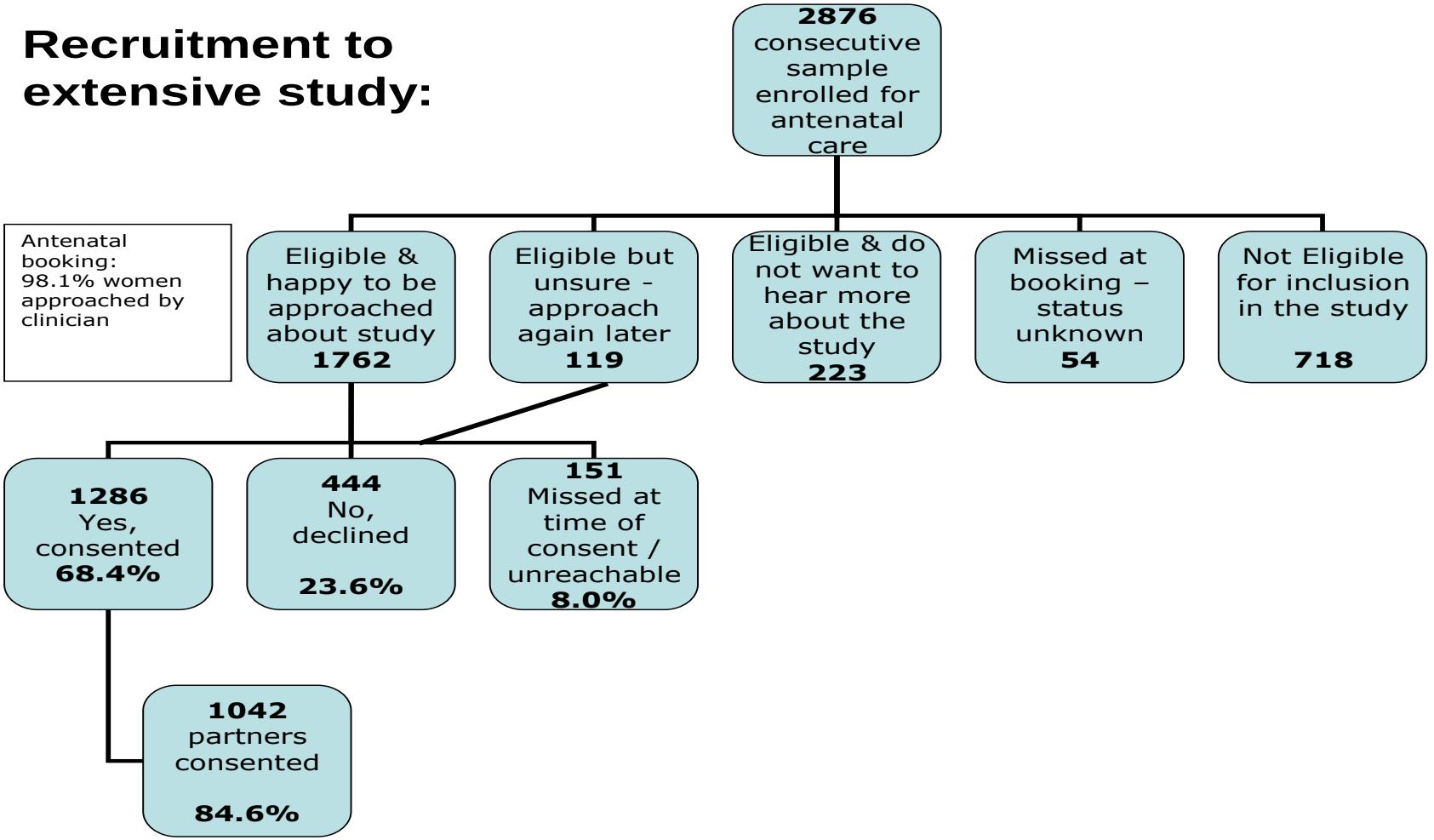


Figure 3 Intensive study: Recruitment and attrition to Phase 2

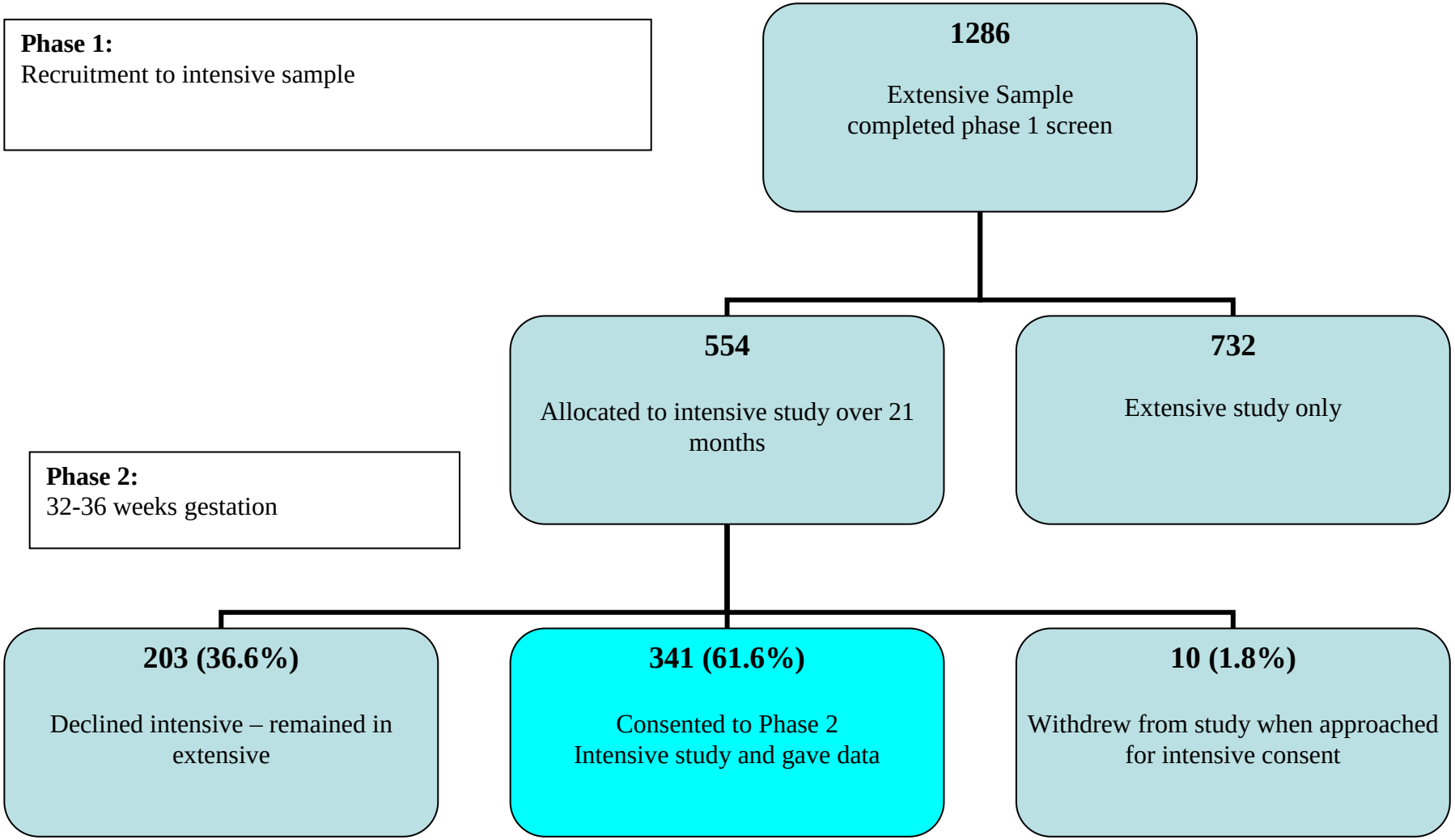
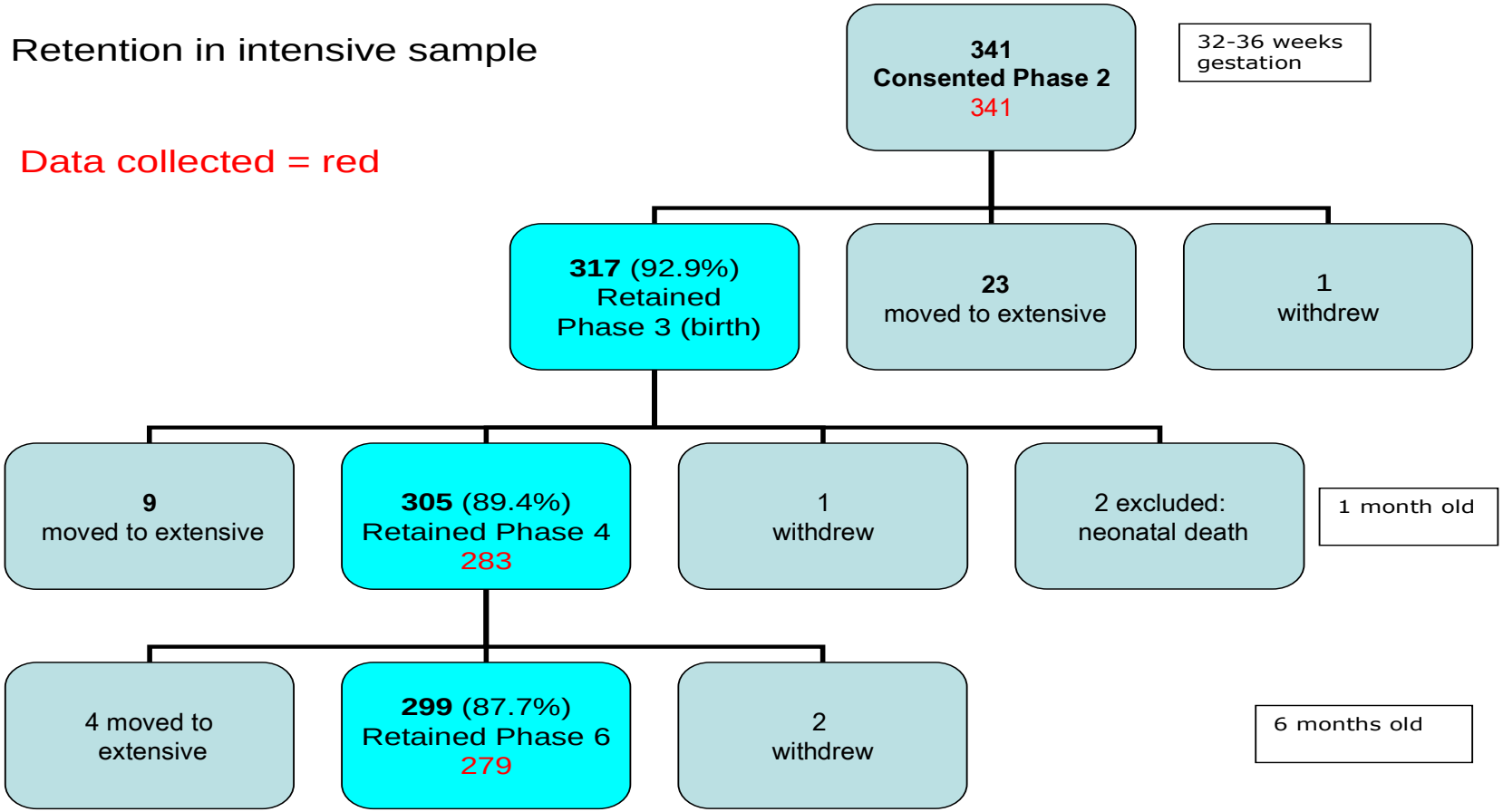


Figure 4 Intensive study: Attrition to Phase 6



2.4.4 The sample for the current thesis

The sample for the current thesis was a convenience sample drawn from the first 150 intensive participants for whom salivary cortisol testing had been completed from phase 6. Samples were sent periodically to the laboratory for assaying depending on the workload of the technicians there. Samples were sent in a pseudo-random order without systematic bias and did not conform to a consecutive series within the whole WCHADS intensive sample. During the period of study ratings of sensitivity were available from 127 mothers. 91 cases had complete data from all antenatal assessments and postnatal measures for this thesis. The sample used in this study was compared to the remaining cases in the WCHADS intensive series on the basis of demographic composition. Those for whom data was available did not differ from the remaining WCHADS sample in terms of maternal age ($t(334) = 0.39$. $p > .05$) nor did they differ on age leaving education $t(197.86) = 1.23$, $p > .05$). The mean age of women in the present sample was 27.12 (SD 5.93) compared with 27.42 (SD 6.19) in the remaining intensive sample. Mean age leaving education was 18.70 (SD 2.57) in the present sample and 19.12 (SD 3.18) in the remaining sample. When the present sample was compared to the remaining cases in the WCHADS intensive series on levels of deprivation they did not differ significantly (χ^2 (1) = .33, $p > .05$). Within the present sample, 37.4% were in the most deprived category and 62.6% were not. Within the remaining sample, 40.8% were living in the most deprived conditions and 59.2% were not. The lack of difference between the present sample and the remaining sample suggests that the sample reported in the current thesis is representative of the WCHADS intensive sample.

2.5 Inclusion and exclusion criteria

Only primiparous women who would be over the age of 18 at their 20 week scan were approached for consent. Late attendance for antenatal care is associated with relationship dysfunction in pregnancy (McFarlane et al., 1997) therefore anyone who registered late for antenatal care during this period was still invited to be part of the study. No exclusions were made for low birth-weight (<2500g) or preterm delivery (<37 weeks) as antenatal stress is known to be associated with both of these birth outcomes. Severe relationship dysfunction also associated with prematurity and low

birth-weight (Altarac & Strobino, 2002) which also constitute risks for developing later conduct problems in childhood. Women were excluded from further study after delivery if their infant did not survive or was born with severe congenital or developmental abnormalities.

2.6 Procedures

2.6.1 Antenatal and birth outcome

Phase 1 (20 weeks gestation)

All women had expressed interested in the study at 12 weeks were approached by research midwives at their 20 week scan. Informed consent was gained for inclusion in the 'extensive' study which included phases 1,3,5 and 7 of data collection for the WCHADS (see appendix 4a for consent forms and information sheets). As part of this initial consent process women were informed that women who were experiencing a lot of stress, and some who were not, would be invited to be part an intensive part of the study. They were made aware that if selected as part of the 'intensive' sample they would be asked to give a separate written informed consent. Permission was gained for a researcher on the study to contact the woman during the third trimester of pregnancy (Phase 2) should she be selected to be part of the 'intensive' sample.

Once informed consent had been given, research midwives administered the Phase 1 interview, and a set of questionnaires covering current relationships, recent mental and physical health and demographic information (see measures).

Any women who did not consent to take part in the study were asked for basic demographic information (age and post code) in order to establish any differences in socio-economic status between the sample who agreed to take part and those who declined.

Phase 2 (32 weeks gestation)

Women selected for the 'intensive' study were contacted by a researcher who arranged a meeting to explain the study procedure. They were provided with detailed information sheets and given the opportunity to ask questions (see appendix 4b for consent forms and information sheets). If in agreement, written informed consent to be part of Phase 2 and 4 was given and a time to conduct the interview was planned.

Interviews were conducted in private at the WCHADS study base or at the women's home according to her preference. The interview assessed multiple aspects of psychological and social functioning. Antenatal measures of depression and anxiety symptom levels were included and are the focus of the current study at this phase.

Phase 3 – Perinatal

Obstetric and neonatal outcome information, namely birth-weight, gestational age and any delivery complications, was obtained from women's hospital records after delivery.

2.6.2 Postnatal

Phase 6 (6 months postnatal)

Before the infant reached six months of age, the Phase 2 interviewer contacted the mother to invite her and her baby to come to the WCHADS study base for the assessment. On arrival in the laboratory, time from last food or drink and time from last awaking, along with any use of steroid creams were recorded on the observation sheet. The researcher then outlined the structure of the assessment and provided a detailed information sheet, allowing the mother the opportunity to ask questions before written consent forms were signed (see appendix 4c for consent forms and information sheets). If the baby had been awake for over 30 minutes and not had any food or drink (other than water) in this time, the baseline saliva sample was then taken (see infant measures) to be used for cortisol analysis. If appropriate, other non-stressful tasks were completed whilst sufficient time had passed since the infant's last sleep, food or drink intake. Phase 6 comprised a 2-3 hour laboratory assessment, including breaks which were timed according to the mother or infant's needs. Multiple measures were completed that relate to other lines of enquiry in the larger study. Of central importance to the current study were the procedures for collecting saliva samples for cortisol analysis and the procedures for observing mother-infant interaction from which maternal sensitivity codes were derived. Each will be described in turn.

Infant cortisol levels were assessed from saliva samples taken at baseline (described above) and 20 minutes post a social stressor, termed the still-face procedure. This

procedure began with the infant being seated in a high chair facing the mother at eye-level, approximately 1 metre away. Mothers were instructed to interact with their babies as they would at home during the engagement episodes. For the still-face episode, the instruction was to hold her face still, with a neutral expression for 2 minutes, looking slightly above the infant's head to avoid eye contact and not to touch the baby. The beginning and end of each episode was indicated by a knock at the door by the researcher. The time of reengagement was noted down by the researcher so as to be ready 20 minutes later to do the post-stressor saliva sample from the infant. More detail on this procedure is given in Measures section 2.7.2.

Quality of mother-infant interaction was observed during an 8 minute period of playful interaction with her infant. A camcorder was used to record the interaction between mother and infant. Mothers were asked to 'play with as you might normally do with your baby' with a standardized set of toys. This followed a period of 7 minutes recorded whilst the dyad played with a non-standardized toy brought in by the mother. The end of the first 7 minutes was indicated by a knock at the door. The recordings were later rated for maternal sensitivity (see maternal measures). The current study focuses on data derived only from the 8 minute interaction because of the standardization of the procedure across dyads.

2.7 Measures

2.7.1 Maternal measures

Demographics

Demographic information was obtained as part of the questionnaire pack at Phase 1, including marital and employment status, age when left full-time education, ethnic origin and socioeconomic deprivation derived from postcode data using the IMD (Office for National Statistics, 2004). IMD scores were converted into categories, with 1 being 'most deprived' and 5 being 'least deprived' for the purpose of analysis.

Smoking and alcohol use

Women were asked to report any smoking or alcohol use during pregnancy on categorical 4-point scales as part of the Phase 1 and 2 questionnaires. For data

analysis the scales were collapsed to a binary category indicating presence or absence of these behaviours during pregnancy.

The Dunedin Relationship Scale (Moffitt, Caspi, Krueger, Magdol, Margolin, Silva & Sydney, 1997)

The Dunedin Relationship Scale was constructed to measure both physical and psychological abuse within intimate partner relationships. The physical abuse scale uses 9 items from a previously established measure of partner violence, the Conflict Tactics Scale (CTS) (Straus, 1990) plus four additional items. The psychological abuse scale consists of 20 statements describing controlling, demeaning or other abusive behaviours, 2 of which were taken from the CTS. The scale is administered in a face-to-face interview where participants are instructed to record "yes" or "no" on a sheet of paper to indicate whether the each behaviour has occurred in the past 12 months. This format ensures privacy whilst overcoming literacy problems. Each item is administered twice; first the participant reports their behaviour towards their partner then their partner's behaviour towards them. "Variety" scores are used in the scale to signify how many acts of abuse have occurred in the specified time period rather than frequency ratings. Scoring in this manner is methodologically superior as the data is more reliable, not as skewed and it prevents less severe, frequently occurring acts (e.g. shove or grab) being given more weight than rare but serious behaviours (e.g. threatening with a knife). The number of different abusive acts has been found to correlate highly with other severity measures of abuse (Elliott & Leverton, 2000). The psychological abuse scale has good internal reliability when participants reported their own acts of abuse towards their partner (Cronbach's alpha = 0.84) and their partner's psychological abuse towards them (alpha = 0.87) (Moffitt et al., 1997). The DRS was administered during the Phase 1 interview and scores were used to select the participants for the 'intensive' sample that is the focus of this thesis.

The Edinburgh Postnatal Depression Scale (J. Cox, Holden & Sagovsky, 1987)

The Edinburgh Postnatal Depression Scale (EPDS) is self-report scale designed as a screening tool for depression and has been used extensively during pregnancy and with postnatal women (Cox & Holden 2003). It was developed to address the clinical concern of failure to identify and therefore treat depression in the peripartum. Depression in this period is common with 10-15% of mothers experiencing marked

illness (Watson, Elliott, Rugg & Brough, 1984). Postnatal depression may have serious consequences for the children of these women as it has been associated with childhood behaviour disturbances at three years (Wrate, Rooney, Thomas & Cox, 1985). Existing depression screen were found to lack sensitivity and specificity in the postnatal period (Cox et al. 1997) possibly due to the emphasis on somatic symptoms that may exist as part of the normal physiological response to pregnancy and childbirth. The EPDS is a 10-item scale requiring women to respond to questions about emotional health. Responses are scored from 0-3 and summed to yield a total score. It is simple and takes 5 minutes to complete, making it acceptable to mothers and non-specialist health workers. The reported sensitivity, or proportion of true positives detected by the EPDS, ranges from 86-100% and the specificity, or number of true negatives, ranges from 78%-89% with a positive predictive value of 83% (Cox et al., 1987). Cox et al. (1987) suggest a cut of score of 12/13 indicates women who are most likely to be suffering from depressive illness but advise a threshold of 9/10 for further assessment if the scale is being used routinely in the primary care setting. Further research validates these cut off scores as indicating 'probably depression' and 'possible depression' respectively (Elliott & Leverton, 2000). In this study, the EPDS was used as a continuous measure of depression symptomatology in the 2nd and 3rd trimesters of pregnancy (Phase 1 and 2) and at 6 months postnatally (Phase 6).

The State-Trait Anxiety Inventory (Spielberger, 1983)

The State-Trait Anxiety Inventory (STAI) is a self-report measure consisting of two sets of twenty statements describing how the participant feels which are rated on a four point intensity scale. One set of statements describe how the participant feels in general and measure frequency and intensity of anxiety over time (trait anxiety) which is thought to be a relatively stable personality trait. The other set of statements ask the participant to rate how they feel at a particular moment in time (state anxiety). State anxiety fluctuates depending on whether the individual perceives the situation they are in to be threatening and is a measure of the effect of a stressor on the individual. People with high trait anxiety perceive more situations as threatening and also tend to have high state anxiety scores.

The STAI has been used widely with both pregnant (Grant et al., 2009; Van den Bergh & Marcoen, 2004) and non pregnant samples and has good internal consistency with Cronbach's alpha coefficients reported at .92 (Spielberger, 1983) and .95 in a recent study into prenatal anxiety (Grant et al., 2009). In the present study, the STAI was administered in the 2nd and 3rd trimesters of pregnancy (Phase 1 and 2) and at the 6 month postnatal assessment (Phase 6).

Maternal sensitivity

Videotapes of the 8 minute play episode were rated for sensitivity of the mother-infant interaction with the Mother-Infant Interaction Global Ratings Scale (Owen, 1992 as cited in McElwain, 2006). This 5 point qualitative scale is based on the work of Ainsworth et al. (1978) and has been used extensively in the maternal sensitivity literature, especially by the National Institute of Child Health and Human Development (NICHD) Early Child Care Research Network (NICHD Early Child Care Research Network, 1999) . Aspects of the mother-infant interactions were rated on 5-point scales (1=*not characteristic* to 5=*highly characteristic*). Mothers were deemed more sensitive if they provided appropriate, contingent responses to their infant's signals. Interactions were rated on the quality of the following maternal behaviours: sensitivity to distress and non-distress and a global rating of sensitivity, intrusiveness, detachment, positive and negative regard for the infant, animation and stimulation of development. For the present study the global rating of sensitivity will be used. Tapes were randomly allocated to the three coders using a random number generator. All three coders were blind to the mother's level of relationship discord and her levels of antenatal anxiety.

2.7.2 Infant measures

The still-face procedure

The still-face paradigm (Tronick, Als, Adamson, Wise & Brazelton, 1978) was designed to assess the infants' expressive modalities and how they are related to variations in mother-infant interaction in order to learn more about infants' emotional experiences. The procedure consists of three, 2-minute episodes: normal engagement episode, a still-face episode in which the mother becomes unresponsive and a reengagement of normal interaction episode. The still-face episode is known to

produce behavioural changes in infants including increased negative affect and gaze aversion and decreased smiling compared to the normal interaction episode. Physiological changes have also been demonstrated by an increase in cortisol levels from baseline to after the SFP (Hayley, Handmaker & Lowe, 2006; Hayley & Stansbury, 2003; Grant et al., 2009). It has been widely used as a social stressor paradigm to evaluate infant regulatory capacities (for a thorough review see (Mesman, van IJzendoorn & Bakermans-Kranenburg, 2009)). Field et al. (2004) explain the 'still-face effect' in terms of the infants' use of the mother as a facilitator of emotion regulation. During the normal interaction, the mother provides an appropriate level of stimulation to maintain optimum infant arousal. When the mother ceases to be available the infants' emotions become dysregulated due to the lack of maternal regulatory input resulting in the observed behavioural and physiological changes.

Saliva samples

Two saliva samples were collected from each infant to capture their cortisol response to the still-face procedure. The first was collected shortly after arrival at the study base to establish a baseline level and the second, the post-stressor sample, was taken 20 minutes after the start of the reengagement episode of the still-face procedure. Saliva was collected from the infant's mouth using an absorbent cotton wool roll. The researcher held the cotton wool roll tightly at one end whilst allowing the infant to suck on it and mopped up and saliva along the lower teeth line, in the cheek cavities and from the chin. There was no time limit on collection of the sample. When the swab was sufficiently wet it was transferred into a plastic tube and put into the freezer as soon as possible, where it was stored until assayed. No oral salivary stimulants were used to avoid contamination of the sample. Time of sample collection was recorded, along with time of last awakening and feeding and the use of any steroid based medicines or creams. Salivary cortisol was assayed using a standard immunoassay (Salimetrics Europe, UK). The inter-and intra-assay coefficients of variance were 7.9% and 8.9% respectively.

2.8 Inter-rater reliability

Inter-rater reliability within the maternal sensitivity literature is typically high. When assessed using intraclass correlations, reports of 80% to 85% level of agreement

between independent coders are commonplace. For the current study, 2 of the coders were trained to reliability by Dr Owen from the NICHD. A set of 15 randomly selected tapes were separately coded by Dr Owen and the two research assistants from the WCHADS using the standardized manual of instructions. A third rater was then trained by the other 2, and inter-rater reliability between the 3 WCHADS raters was established using the same set of 15 randomly selected tapes. This two step training procedure was adopted because Dr Owen resides in the USA so was unable to train the third rater who joined the WCHADS research team after the initial training took place. Inter-rater reliability was calculated using one-way analysis of variance to derive intra-class correlations which ranged between 0.81 and 0.91 representing a high level of agreement and are reported in table 2.

Table 2 Inter-rater agreement (intra-class correlations) for Phase 6 maternal sensitivity data.

	Global Sensitivity (8 minutes)
Rater 1 vs. rater 2	0.90
Rater 1 vs. rater 3	0.81
Rater 2 vs. rater 3	0.91

3. Results

3.1 Mother and infant sample characteristics

The demographic characteristics of the 91 mothers and their infants who are the focus of this thesis are reported in Tables 3.1 and 3.2. The majority of the participants were white British and were either married or cohabiting with their partners. Most women were employed on a full or part time basis at the time of Phase 1 recruitment in mid-pregnancy although almost half of the sample was living in the most deprived socioeconomic circumstances according to the government Indices of Multiple Deprivation (IMD). Proportions of female and male infants were similar. None of the babies were born prematurely (prior to 37 weeks) and there were no infants born with low birth weights (<2500g) in the current study.

Table 3.1 Maternal Characteristics at Phase 1 recruitment

	Mean (SD)	
Demographics		
Age (years)	27.12 (5.93)	
Education (age finished)	18.67 (2.56)	
Number of weeks pregnant at:		
Phase 1 recruitment	20.66 (2.15)	
Phase 2 consent	32.67 (1.90)	
	N	%
Ethnicity		
White	88	(96.7)
Other	3	(3.3)
Deprivation per IMD quintile		
1 (most deprived)	34	(37.4)
2	15	(16.5)
3	20	(22.0)
4	7	(7.7)
5 (least deprived)	15	(16.5)
Marital Status		
Married	35	(38.5)
Single	11	(12.1)
Separated	1	(1.1)
Cohabiting	33	(36.3)
Partner living elsewhere	11	(12.1)
Employment		
Full time paid	67	(73.6)
Part time paid	9	(9.9)
Self employed	2	(2.2)
Unemployed	8	(8.8)
Sick leave or disability	1	(1.1)
Full time education/training	1	(1.1)

Part time education/training	1	(1.1)
Voluntary work	1	(1.1)
Full time education & part time work	1	(1.1)
Smoking		
Never	51	(56)
Before pregnancy only	11	(12.1)
Before and during pregnancy	29	(31.9)

Table 3.2 Infant Characteristics

	Mean	(SD)
Obstetric outcomes		
Gestational age at birth (weeks)	40.15	(1.22)
Birth weight (g)	3395.09	(512.52)
Infant characteristics at Phase 6		
Age (weeks)	28.79	(3.14)
	N	%
Sex		
Male	40	44
Female	51	56

3.2 Approach to statistical analysis

Data analysis was completed using SPSS for Windows (version 15). Final analyses were confined to participants who had complete data sets for the variables of interest (n = 91).

The distributions of the predictor variables and outcome measures were examined for departures from normality using histograms and measures of skewness and kurtosis. Transformations were applied to all non-normally distributed data to ensure that the assumptions underlying the use of parametric tests were fulfilled. Scores on the anxiety and depression symptoms scales were negatively skewed, so log transformations were computed to provide acceptable skewness and used in subsequent analyses (see appendix 1 for histograms of raw and transformed values). Logarithmic transformation is an effective way of reducing skewness because it pulls outlying data from the tails of the distribution to its centre. Where simple log transformations were not effective, the approach was to use modified log transformations according to the model ln(x + score), where "x" represents the mean of the values that added to the corresponding scores led to zero skewness. The values

used to calculate the mean x were identified by employing the zero-skewness command in the statistical programme STATA. These mean values were revealed as +7.4 for depression and -15.87 for the state anxiety scores. Distributions for the raw cortisol data were also skewed, so log transformations of pre and post test values were computed and used in subsequent analyses (see appendix 2 for histograms of raw and transformed values)

Associations within and between the different demographic (age, education and deprivation), obstetric (smoking and birth weight), maternal stress and sensitivity predictors were examined first to ensure any underlying relationships were understood, prior to their use in hypothesis testing proper. Prenatal predictor variables were examined as continuous variables as opposed to categorical indices of high versus low symptoms scores in most analyses. Bivariate associations between continuous variables were examined using Pearson's r with two-tailed significance testing. Independent t-tests were used to determine associations between continuous and categorical variables. Cross tabulation with Chi squared analysis was used to explore relationships between two categorical variables.

Stepwise multiple regression models were constructed to examine whether indices of maternal stress during pregnancy accounted for a significant variance in infant cortisol outcomes after accounting for important confounding variables. In view of the contrasting approaches taken in the literature, analyses were repeated to examine prediction of infant baseline cortisol levels and cortisol reactivity in turn. Demographic variables that had made a significant contribution to the model as it was built, or for which there was substantial evidence in the literature of a possible association with either one of the other predictor variables or one of the outcomes, were retained in the model in order to control for their effects. Prior to the regression analysis collinearity of data had been checked to ensure variables within the regression model were too not highly correlated with one another. Separate models were constructed to examine the influence of depression on outcome separately from the effects of anxiety as these variables were highly correlated. All other coefficients between predictor variables were small indicating that there was no collinearity in the data and so the data was suitable for regression analysis.

Multiple comparisons were made within the course of the analyses. Although it was recognised that multiple comparisons can increase the likelihood of Type 1 errors, a decision was taken not to apply a conservative approach as the current study is one of very few of its kind and the field of knowledge is in its infancy. In a small sample, a moderate effect which may be clinically meaningful might be missed if the focus of the study findings relied on statistical significance alone. Taking a conservative approach may increase the likelihood for Type 2 errors, where the null hypothesis is accepted erroneously. The magnitude of associations between variable are reported throughout so as to enable interpretation of likely effect sizes.

3.3 Bivariate associations

3.3.1 Associations within demographic and maternal stress predictor variables

Associations within demographic and maternal stress variables were examined first. Maternal age was moderately and significantly associated with age on finishing education, ($r = .33$, $p < .01$). Pre and post natal mood scores were strongly associated with each other demonstrating continuity in mood over time (see Tables 4 and 5). Anxiety and depression scores were strongly associated at Phase 1 ($r = .52$, $p < .01$), Phase 2 ($r = .58$, $p < .01$), and Phase 6 ($r = .59$, $p < .01$). The strength of these associations indicates high levels of conceptual overlap or comorbidity. Both depression and anxiety are used in the literature as indices of prenatal stress. In order to enable cross-study comparability, each will be examined in separated analyses in this thesis to test a priori hypotheses. The strong correlation between the STAI and EPDS scores prevents them from being entered into the same regression model to assess the effect of one whilst controlling for the effect of the other.

Table 4 Associations within maternal prenatal and postnatal anxiety scores.

	Prenatal		Postnatal
	Phase 1 Anxiety	Phase 2 Anxiety	Phase 6 Anxiety
Phase 1 Anxiety	1	-	-
Phase 2 Anxiety	.41**	1	-
Phase 6 Anxiety	.33**	.39**	1

** Pearson correlation is significant at the 0.01 level (2-tailed)

Table 5 Associations within maternal prenatal and postnatal depression scores.

	Prenatal		Postnatal
	Phase 1 Depression	Phase 2 Depression	Phase 6 Depression
Phase 1 Depression	1	-	-
Phase 2 Depression	.65**	1	-
Phase 6 Depression	.52**	.55**	1

** Pearson correlation is significant at the 0.01 level (2-tailed)

3.3.2 Associations between maternal demographic and mood variables

Bivariate associations between maternal demographic variables and prenatal and postnatal anxiety and depression scores were examined. Results are summarised in Table 6. Maternal age was significantly and negatively correlated with prenatal depression but not postnatal depression or anxiety at any time point. The magnitude of correlations was small to moderate.

Table 6 Associations (Pearson's r) between maternal demographic variables and anxiety and depression scores at Phases 1, 2 and 6.

	Phase 1 Anxiety	Phase 2 Anxiety	Phase 6 Anxiety	Phase 1 Depression	Phase 2 Depression	Phase 6 Depression
Maternal Age	.03 (.77)	-.13 (.24)	-.04 (.70)	-.29 (.01)*	-.21 (.05)*	-.13 (.24)
Age finished education	-.19 (.08)	-.10 (.35)	.01 (.90)	-.18 (.09)	-.10 (.33)	-.18 (.10)

* Significance levels are given in parentheses

Bivariate associations between maternal mood indices and deprivation were examined using t-tests to compare mean scores. Table 7 gives the means and standard deviations for the deprived and non-deprived groups for each mood measure at each time point. No significant associations were found for either prenatal or postnatal mood and deprivation.

Table 7 Associations between maternal mood indices and deprivation

	Groups		Significance	Effect size
	Deprived Mean (SD)	Non-deprived Mean (SD)		
Phase 1 Anxiety	2.51 (.79)	2.61 (.71)	t(89) = 0.59, p > .05	.06
Phase 2 Anxiety	2.61 (.59)	2.79 (.57)	t(89) = 1.42, p > .05	.15
Phase 6 Anxiety	2.59 (.60)	2.50 (.62)	t(89) = -0.65, p > .05	.07
Phase 1 Depression	2.84 (.29)	2.74 (.31)	t(89) = -1.44, p > .05	.15
Phase 2 Depression	2.77 (.30)	2.77 (.30)	t(89) = -0.01, p > .05	.00
Phase 6 Depression	2.59 (.31)	2.52 (.34)	t(89) = -0.94, p > .05	.10

3.3.3 Associations between maternal demographic variables and maternal sensitivity

Global sensitivity scores for the 8 minute playful interaction are shown in Table 8. The sensitivity data was dichotomised at the mean score to derive groups in which mothers were then classified as either high or low sensitivity following Grant et al. (2009). The mean scores were 2.52 (SD = .63) for the low sensitivity group and 4.47 (SD = .50) for the high sensitivity group (t(89) = -16.3, p < .001). The high or low sensitivity categorical variable was used in all subsequent analyses involving maternal sensitivity.

Table 8 Sensitivity ratings

Level of sensitivity	N (%)
Not sensitive at all	3 (3.3)
Minimally sensitive	14 (15.4)
Somewhat sensitive	25 (27.5)
Moderately sensitive	26 (28.6)
Highly sensitive	23 (25.3)

Bivariate associations between maternal demographic variables and maternal sensitivity grouping were examined using t-tests to compare mean scores. Table 9 gives the means and standard deviations for the high and low sensitivity groups for each demographic variable. Highly sensitive mothers were significantly older than mothers in the low sensitivity group and were older when they finished education than mothers in the low sensitivity group. Associations between maternal sensitivity and deprivation were not significant χ^2 (1) = 2.07, p > .05. Within the low sensitivity group, 45.2% of women were living in the most deprived conditions and 54.8% were not. Within the highly sensitive group, 30.6% of women were living in the most deprived conditions and 69.4% were not.

Table 9 Associations between demographic variables and maternal sensitivity

	Groups		Significance	Effect size
	Low Sensitivity Mean (SD)	High Sensitivity Mean (SD)		
Maternal Age at Phase 1	24.14 (4.95)	29.67 (5.52)	$t(89) = -4.99$, $p < .01$	.47
Age Finished Education	17.93 (2.41)	19.37 (2.54)	$t(89) = -2.76$, $p < .01$	.28

3.3.4 Associations between indices of mood and maternal sensitivity

Bivariate associations between maternal mood indices and maternal sensitivity were examined. Table 10 gives the means and standard deviations for the high and low sensitivity groups for each mood measure at each time point. There was a significant association between depression at Phase 1 and maternal sensitivity grouping

Table 10 Associations between maternal mood indices and sensitivity

	Groups		Significance	Effect size
	Low sensitivity Mean (SD)	High sensitivity Mean (SD)		r
Phase 1 Anxiety	2.54 (.82)	2.60 (.67)	t(89) = -0.35, p > .05	.04
Phase 2 Anxiety	2.81 (.58)	2.64 (.58)	t(89) = 1.38, p > .05	.14
Phase 6 Anxiety	2.49 (.63)	2.57 (.59)	t(89) = -0.64, p > .05	.07
Phase 1 Depression	2.85 (.29)	2.72 (.31)	t(89) = 2.09, p < .05	.22
Phase 2 Depression	2.81 (.28)	2.74 (.31)	t(89) = 0.17, p > .05	.02
Phase 6 Depression	2.56 (.31)	2.53 (.35)	t(89) = 0.39, p > .05	.04

3.3.5 Associations within obstetric risks and with maternal mood and sensitivity

Bivariate associations between smoking during pregnancy, birth weight and maternal mood variables were examined using t-tests to compare mean scores. Table 11 gives the means and standard deviations for anxiety and depression scores at each time point for smoking and non-smoking groups during pregnancy groups. Women who smoked during pregnancy had significantly higher depression scores at all three time points. Associations between smoking and anxiety were weaker and fell short of conventional statistical significance. However, these effect sizes indicated that the group difference may have achieved significance in a larger sample. There was no significant difference in mean infant birth weight between groups who smoked or did not smoke during pregnancy. Again, the size of group differences indicated an effect

size of 0.40 SD which may achieve criterion level for statistical significance in a larger sample.

Table 11 Mean maternal anxiety and depression scores and birth weight comparing 'smoked during pregnancy' and 'didn't smoke during pregnancy' groups

	Smoking during pregnancy		Significance	Effect size
	No Mean (SD)	Yes Mean (SD)		
Phase 1 Anxiety	2.48 (.72)	2.77 (.75)	t(89) = -1.76, p > .05	.18
Phase 2 Anxiety	2.69 (.59)	2.79 (.58)	t(89) = -0.75, p > .05	.08
Phase 6 Anxiety	2.47 (.58)	2.67 (.65)	t(89) = -1.45, p > .05	.15
Phase 1 Depression	2.69 (.27)	2.97 (.29)	t(89) = -4.45, p < .001	.43
Phase 2 Depression	2.69 (.29)	2.94 (.25)	t(89) = -4.05, p < .001	.39
Phase 6 Depression	2.45 (.29)	2.74 (.35)	t(89) = -4.17, p < .001	.40
Birth weight	3458.68 (519.90)	3259.14 (476.82)	t(89) = 1.75, p > .05	.18

Correlation between birth weight and prenatal depression and anxiety scores were examined. Birth weight was not significantly correlated with anxiety or depression at either time point (all p values > .05). The magnitude of correlations are summarized in Table 12.

Table 12 Pearson correlations (p value) between transformed prenatal anxiety and depression scores and birth weight

	Phase 1 Anxiety	Phase 2 Anxiety	Phase 1 Depression	Phase 2 Depression
Birth weight	-.09	-.00	-.13	.02
	(.41)	(.98)	(.23)	(.87)

3.3.6 Associations between obstetric risks and maternal sensitivity

Bivariate associations between birth weight and maternal sensitivity were examined first and no significant association was found. Mean birth weight in the low sensitivity group was 3344.31g (576.20) and it was 3438.61g (452.49) in the high sensitivity group. There was no significant difference between the groups ($t(89) = -0.87$, $p > .05$, $r = .09$).

Associations between smoking and maternal sensitivity were also not significant $\chi^2 (1) = 2.66$, $p > .05$. Within the low sensitivity group, 40.5% of women smoked during pregnancy and 59.5% did not. Within the highly sensitive group, 24.5% of women smoked during pregnancy and 75.5% did not. Based on the odds ratio, the odds of women smoking during pregnancy were 2.10 times higher if they were in the low sensitivity group.

3.3.7 Summary of bivariate analyses

Bivariate analyses revealed that all the primary predictors were associated with at least one other predictor variable, with the exception of deprivation and birth weight. These associations within and between predictor variable could therefore explain any relationship found between candidate predictors and outcomes. It is important to control for these potential confounding variables in subsequent model building. Given the reduction in statistical power associated with entering multiple predictors are entered into a regression model a decision was made to leave deprivation out of subsequent analyses. Although birth weight was not significantly associated with any

other predictor variables in this sample, there was a negative relationship with smoking (r = .18). Since there are associations between maternal stress and birth weight, and birth weight and negative infant outcome in the literature a decision was made to retain birth weight in subsequent analyses.

3.4 Cortisol analysis

Cortisol values were screened for outliers, defined as any value 3 SD above or below the mean (Grant et al., 2009; Schuetze et al., 2008; Ramsay et al., 2003). Following Grant et al. (2009), outliers were winsorized meaning that the extreme values were replaced with the value corresponding to 3 SD above the mean. For pre test cortisol M = 4.0 (SD 3.2); for post test cortisol M = 4.3 (SD 3.7). There were no values 3 SD or more below the mean. Given that the current sample was selected to have full data, a statistic pertaining to proportional loss of cortisol data cannot be reported for this sample. However, out of the whole intensive sample (n = 276), 88.0% of infants gave both saliva samples, 9.1% gave only one sample and 2.9% were not able to give any samples due to distress. No infants were currently using cortisol based creams at the time of testing.

3.4.1 Associations between baseline cortisol values and cortisol change scores

Following Brennan et al. (2008), associations between baseline cortisol and cortisol reactivity (indicated by the pre to post stressor change in cortisol level) were explored using Pearson's r two tailed tests. Cortisol reactivity was negatively correlated with baseline values ($r = -.46$, $p < .01$). This is consistent with Brennan et al. (2008) who found a similar correlation (r = -.47) and confirmed the Wilder's (1958) Law of Initial Value. All subsequent analyses examining cortisol reactivity therefore include baseline cortisol as a covariate.

3.4.2 Timing of cortisol collection

Laboratory visits were conducted on weekdays and scheduled throughout the day (9 a.m. to 4 pm) in order to fit in with mothers' and infants' daily routines. Correlations between time of day and pre and post stressor cortisol levels were computed (see

Table 13). The time of day the baseline (pre still-face) cortisol was taken was correlated with the post still-face cortisol value, although the magnitude of the association was small to moderate. The time of post still-face cortisol sampling correlated with post still-face cortisol value to a similar degree. Paradoxically, there was no significant correlation between the time of day the baseline was taken and the baseline value. This apparent lack of association between time of day and baseline cortisol levels is consistent with other studies, Grant et al. (2009) found all correlations to be non significant. However, to err on the side of caution, the time of day was controlled for in subsequent analyses as there was a significant association was found within the present data.

Table 13 Correlations between transformed cortisol values before and after the still-face (SF) and time of collection

	Pre SF Cortisol	Post SF Cortisol	Pre SF Time	Post SF Time
Pre SF Cortisol	1	-	-	-
Post SF Cortisol	.43**	1	-	-
Pre SF Time	.01	-.21*	1	-
Post SF Time	.00	-.20	.99**	1

* P < .05, *** P < .001

3.5 Hypothesis testing – hypothesis 1

Hypothesis 1: High levels of prenatal maternal anxiety or depression will be associated with increased infant basal cortisol levels and increased cortisol reactivity to a social stressor. These effects will be evident after controlling for possible demographic confounds (maternal age and education), smoking behaviour in pregnancy, birth weight and timing of cortisol sampling. The timing of stress during pregnancy will have an effect on the cortisol outcomes.

3.5.1 Combined contribution of all confounding variables to prediction of infant cortisol

Before hypothesis testing proper was commenced, a two step linear regression analysis was conducted to examine the combined association between maternal demographic variables (age and education), smoking and birth weight and infant baseline cortisol at 6 months. The baseline cortisol score was the dependant variable and the time of day that the baseline cortisol was sampled was entered as a first step because of the relationship between time of day and cortisol levels described above. In the second step, the maternal demographic variables, smoking and birth weight were entered into the model. Overall, the model was not significant, ($F(5,85) = 0.89$, $p > .05$). Entering the confounding variables at step 2 accounted for 5% of the variance in baseline cortisol but did not make a significant contribution to the model.

The regression analysis was then repeated to examine the contribution of all the confounding variables on infant cortisol reactivity. In order to do this an extra step was added to the model to control for baseline cortisol levels. Overall the model was significant, ($F(6,84) = 5.70$, $p < .001$). Step 1, timing of cortisol accounts for 4% of the variance and was a significant contributor to the model. Adding baseline cortisol at step 2 accounted for 21% of the variance and made a significant contribution to the model. None of the confounding variables added at step 3 made a significant contribution to the model and as a group they accounted for 4% of the variance. Tables 14 and 15 summarise the results of the regression analysis.

Table 14 Summary statistics for regression analysis of maternal demographic variables, smoking and birth weight as predictors of infant baseline cortisol

	Standardised β	P value
Step 1		
Pre SF time	.01	.94
Step 2		
Pre SF time	-.02	.87
Maternal Age	-.10	.40
Age finished education	.09	.41
Smoking	.15	.19
Birth weight	.13	.25

Note: $R^2 = .01$ for Step 1, $\Delta R^2 = .05$ for Step 2

Table 15 Summary statistics for regression analysis of maternal demographic variables, smoking and birth weight as predictors of infant cortisol reactivity

	Standardised β	P value
Step 1		
Pre SF time	-.21	.05
Step 2		
Pre SF time	-.21	.03
Baseline cortisol	-.46	.00
Step 3		
Pre SF time	-.21	.04
Baseline cortisol	-.47	.00
Maternal Age	-.12	.21
Age finished education	-.07	.46
Smoking	.06	.59
Birth weight	-.05	.58

Note: $R^2 = .04$ for Step 1, $\Delta R^2 = .21$ for Step 2, $\Delta R^2 = .04$ for Step 3

3.5.2 Associations between maternal stress at Phase 1 and infant cortisol

In order to test the hypothesis 1, 8 separate multiple linear regression analyses were run. The regression models were used to assess the relative contribution made by indices of maternal stress as independent variables in the prediction of variance in subsequent infant baseline cortisol levels. Firstly, the association between prenatal stress at Phase 1 and infant cortisol was examined. This involved 4 regression analyses looking at the effect of anxiety and depression on baseline cortisol and cortisol reactivity (Tables 16-19).

Then the relationship between prenatal stress at Phase 2 and infant cortisol was examined to allow comparison of timing of stress. Again, this will involve 4 regression analyses as anxiety and depression were examined separately in relation to baseline cortisol and cortisol reactivity (Table 21-24).

The prior literature and the bivariate comparisons in the current study suggest that maternal demographic variables, birth weight, maternal smoking during pregnancy, time of day of cortisol collection and baseline cortisol level (when reactivity is the outcome) may influence infant cortisol outcomes either directly or by association with other predictor variables in the study. All these variables were retained and controlled for in the first steps of the regression models.

3.5.3 Associations between Phase 1 maternal stress and infant baseline cortisol

When Phase 1 anxiety was the index of prenatal stress, the overall model did not reach significance ($F(6,84) = 0.79$, $p > .05$). There was no main effect of maternal anxiety at Phase 1 on infant baseline cortisol levels when the influence of the covariates had been controlled for. Entering maternal anxiety into step 3 of the model made no significant contribution and accounted for no further variance (see Table 16).

Table 16 Summary statistics for regression analysis of Phase 1 anxiety as a predictor of infant baseline cortisol

	Standardised β	P value
Step 1		
Pre SF time	.01	.94
Step 2		
Pre SF time	-.02	.87
Maternal Age	-.10	.40
Age finished education	.09	.41
Smoking	.15	.19
Birth weight	.13	.25
Step 3		
Pre SF time	-.02	.89
Maternal Age	-.09	.43
Age finished education	.08	.47
Smoking	.16	.17
Birth weight	.13	.26
Phase 1 Anxiety	-.06	.56

Note: $R^2 = .01$ for Step 1, $\Delta R^2 = .05$ for Step 2, $\Delta R^2 = .00$ for Step 3.

When Phase 1 depression was the index of prenatal stress, the overall model did not reach significance ($F(6,84) = 0.76$, $p > .05$). There was no main affect of maternal depression at Phase 1 on infant baseline cortisol levels when the effect of covariates had been controlled for. Entering maternal depression into step 3 of the model made no significant contribution and accounted for no further variance (see Table 17).

Table 17 Summary statistics for regression analysis of Phase 1 depression as a predictor of infant baseline cortisol

	Standardised β	P value
Step 1		
Pre SF time	.01	.94
Step 2		
Pre SF time	-.02	.87
Maternal Age	-.10	.40
Age finished education	.09	.41
Smoking	.15	.19
Birth weight	.13	.25
Step 3		
Pre SF time	-.02	.89
Maternal Age	-.11	.36
Age finished education	.09	.42
Smoking	.17	.17
Birth weight	.12	.27
Phase 1 Depression	-.05	.68

Note: $R^2 = .01$ for Step 1, $\Delta R^2 = .05$ for Step 2, $\Delta R^2 = .00$ for Step 3.

3.5.4 Associations between maternal mood at Phase 1 and infant cortisol reactivity

When Phase 1 anxiety was the index of prenatal stress, the overall model was significant ($F(7,83) = 4.82$, $p < .001$). There was no main affect of maternal anxiety at Phase 1 on infant cortisol reactivity after the effect of covariates had been controlled for. Entering maternal anxiety into step 4 of the model made no significant contribution and accounted for no further variance (see Table 18).

Table 18 Summary statistics for regression analysis of Phase 1 anxiety as a predictor of infant cortisol reactivity

	Standardised β	P value
Step 1		
Pre SF time	-.21	.05
Step 2		
Pre SF time	-.21	.03
Baseline cortisol	-.46	.00
Step 3		
Pre SF time	-.21	.04
Baseline cortisol	-.47	.00
Maternal Age	-.12	.21
Age finished education	-.07	.46
Smoking	.06	.59
Birth weight	-.05	.58
Step 4		
Pre SF time	-.21	.04
Baseline cortisol	-.47	.00
Maternal Age	-.12	.22
Age finished education	-.07	.46
Smoking	.06	.50
Birth weight	-.06	.58
Phase 1 Anxiety	-.01	.91

Note: $R^2 = .04$ for Step 1, $\Delta R^2 = .21$ for Step 2, $\Delta R^2 = .04$ for Step 3, $\Delta R^2 = .00$ for Step 4.

When Phase 1 depression was the index of prenatal stress, the overall model was significant ($F(7,83) = 4.89$, $p < .001$). There was no main affect of maternal depression at Phase 1 on infant cortisol reactivity after the effect of covariates had been controlled for. Entering maternal depression into step 4 of the model made no significant contribution and accounted for no further variance (see Table 19).

Table 19 Summary statistics for regression analysis of Phase 1 depression as a predictor of infant cortisol reactivity

	Standardised β	P value
Step 1		
Pre SF time	-.21	.05
Step 2		
Pre SF time	-.21	.03
Baseline cortisol	-.46	.00
Step 3		
Pre SF time	-.21	.04
Baseline cortisol	-.47	.00
Maternal Age	-.12	.21
Age finished education	-.07	.46
Smoking	.06	.59
Birth weight	-.05	.58
Step 4		
Pre SF time	-.20	.03
Baseline cortisol	-.47	.00
Maternal Age	-.11	.26
Age finished education	-.07	.47
Smoking	.03	.76
Birth weight	-.05	.62
Phase 1 Depression	-.06	.56

Note: $R^2 = .04$ for Step 1, $\Delta R^2 = .21$ for Step 2, $\Delta R^2 = .04$ for Step 3, $\Delta R^2 = .00$ for Step 4.

3.5.5 Associations between maternal stress at Phase 2 and infant baseline cortisol

When Phase 2 anxiety was the index of prenatal stress, the overall model did not reach significance ($F(6,84) = 0.74$, $p > .05$). There was no main effect of maternal anxiety at Phase 2 on infant baseline cortisol levels when the influence of the covariates had been controlled for. Entering maternal anxiety into step 3 of the model made no significant contribution and accounted for no further variance (see Table 20).

Table 20 Summary statistics for regression analysis of Phase 2 anxiety as a predictor of infant baseline cortisol

	Standardised β	P value
Step 1		
Pre SF time	.01	.94
Step 2		
Pre SF time	-.02	.87
Maternal Age	-.10	.40
Age finished education	.09	.41
Smoking	.15	.19
Birth weight	.13	.25
Step 3		
Pre SF time	-.02	.88
Maternal Age	-.09	.42
Age finished education	.09	.41
Smoking	.15	.20
Birth weight	.13	.25
Phase 2 Anxiety	.03	.81

Note: $R^2 = .01$ for Step 1, $\Delta R^2 = .05$ for Step 2, $\Delta R^2 = .00$ for Step 3.

When Phase 2 depression was the index of prenatal stress, the overall model did not reach significance ($F(6,84) = 0.76$, $p > .05$). There was no main affect of maternal depression at Phase 2 on infant baseline cortisol levels when the effect of covariates had been controlled for. Entering maternal depression into step 3 of the model made no significant contribution and accounted for no further variance (see Table 21).

Table 21 Summary statistics for regression analysis of Phase 2 depression as a predictor of infant baseline cortisol

	Standardised β	P value
Step 1		
Pre SF time	.01	.94
Step 2		
Pre SF time	-.02	.87
Maternal Age	-.10	.40
Age finished education	.09	.41
Smoking	.15	.19
Birth weight	.13	.25
Step 3		
Pre SF time	-.02	.87
Maternal Age	-.10	.39
Age finished education	.09	.41
Smoking	.17	.19
Birth weight	.13	.24
Phase 2 Depression	-.03	.78

Note: $R^2 = .01$ for Step 1, $\Delta R^2 = .05$ for Step 2, $\Delta R^2 = .00$ for Step 3.

3.5.6 Associations between maternal stress at Phase 2 and infant cortisol reactivity

When Phase 2 anxiety was the index of prenatal stress, the overall model was significant ($F(7,83) = 4.83$, $p < .001$). There was no main affect of maternal anxiety at Phase 2 on infant cortisol reactivity after the effect of covariates had been controlled for. Entering maternal anxiety into step 4 of the model made no significant contribution and accounted for no further variance (see Table 22).

Table 22 Summary statistics for regression analysis of Phase 2 anxiety as a predictor of infant cortisol reactivity

	Standardised β	P value
Step 1		
Pre SF time	-.21	.05
Step 2		
Pre SF time	-.21	.03
Baseline cortisol	-.46	.00
Step 3		
Pre SF time	-.21	.04
Baseline cortisol	-.47	.00
Maternal Age	-.12	.21
Age finished education	-.07	.46
Smoking	.06	.59
Birth weight	-.05	.58
Step 4		
Pre SF time	-.20	.04
Baseline cortisol	-.47	.00
Maternal Age	-.12	.22
Age finished education	-.07	.47
Smoking	.06	.59
Birth weight	-.06	.58
Phase 2 Anxiety	-.01	.91

Note: $R^2 = .04$ for Step 1, $\Delta R^2 = .21$ for Step 2, $\Delta R^2 = .04$ for Step 3, $\Delta R^2 = .00$ for Step 4.

When Phase 2 depression was the index of prenatal stress, the overall model was significant ($F(7,83) = 4.82$, $p < .001$). There was no main affect of maternal depression at Phase 2 on infant cortisol reactivity after the effect of covariates had been controlled for. Entering maternal depression into step 4 of the model made no significant contribution and accounted for no further variance (see Table 23).

Table 23 Summary statistics for regression analysis of Phase 2 depression as a predictor of infant cortisol reactivity

	Standardised β	P value
Step 1		
Pre SF time	-.21	.05
Step 2		
Pre SF time	-.21	.03
Baseline cortisol	-.46	.00
Step 3		
Pre SF time	-.21	.04
Baseline cortisol	-.47	.00
Maternal Age	-.12	.21
Age finished education	-.07	.46
Smoking	.06	.59
Birth weight	-.05	.58
Step 4		
Pre SF time	-.21	.04
Baseline cortisol	-.47	.00
Maternal Age	-.13	.21
Age finished education	-.07	.46
Smoking	.06	.58
Birth weight	-.05	.59
Phase 2 Depression	-.01	.90

Note: $R^2 = .04$ for Step 1, $\Delta R^2 = .21$ for Step 2, $\Delta R^2 = .04$ for Step 3, $\Delta R^2 = .00$ for Step 4.

In summary, there was no main effect of mood on baseline cortisol or cortisol reactivity at any time point, once demographics, smoking, time of sampling and baseline cortisol (for reactivity only) had been controlled for.

3.6 Hypothesis testing - hypothesis 2

Hypothesis 2: Maternal sensitive behaviour towards her infant at 6 months will moderate the interaction between maternal prenatal anxiety/depression and infant cortisol levels. High levels of maternal sensitivity will buffer the effect of prenatal anxiety/depression on infant cortisol levels.

In order to examine any main effect of maternal sensitivity on infant cortisol, sensitivity was entered into the model as a fifth step. A sixth step was then added containing an interaction term to explore the influence of maternal sensitivity in interaction with maternal mood on infant cortisol outcome. Entering an interaction term into the multiple regression models required the maternal mood variables to be centred by subtracting each score from the mean. The centred mood variable was used throughout the model. Again, 8 regression models were computed in order to examine sensitivity in relation to Phase 1 and 2 anxiety and depression predicting to infant baseline cortisol and cortisol reactivity (Tables 24-31).

3.6.1 Associations between maternal sensitivity and infant baseline cortisol, alone and in interaction with maternal stress at Phase 1

When Phase 1 anxiety was the index of prenatal stress, the overall model did not reach significance ($F(8,82) = 1.12$, $p > .05$). There was no main effect of maternal sensitivity on infant baseline cortisol levels when the influence of the covariates had been controlled for. Entering maternal sensitivity into step 4 of the model made no significant contribution and accounted for no further variance. Entering the interaction term (maternal sensitivity and Phase 1 anxiety) into step 5 had a significant effect on the model and accounted for 5% of the variance. This indicates that the strength of the prediction from maternal prenatal anxiety at Phase 1 to infant baseline cortisol is moderated by sensitivity (see Table 24) .

Table 24 Summary statistics for regression analysis of maternal sensitivity and the interaction between maternal sensitivity and Phase 1 anxiety as a predictor of infant baseline cortisol

	Standardised β	P value
Step 1		
Pre SF time	.01	.94
Step 2		
Pre SF time	-.02	.87
Maternal Age	-.10	.40
Age finished education	.09	.41
Smoking	.15	.19
Birth weight	.13	.25
Step 3		
Pre SF time	-.02	.89
Maternal Age	-.09	.43
Age finished education	.08	.47
Smoking	.16	.17
Birth weight	.13	.26
Phase 1 Anxiety	-.06	.56
Step 4		

Pre SF time	-.02	.89
Maternal Age	-.09	.48
Age finished education	.08	.49
Smoking	.16	.17
Birth weight	.16	.28
Phase 1 Anxiety	-.06	.57
Sensitivity	.00	.98
Step 5		
Pre SF time	-.03	.80
Maternal Age	-.07	.59
Age finished education	.06	.63
Smoking	.13	.25
Birth weight	-.09	.44
Phase 1 Anxiety	.13	.38
Sensitivity	.01	.97
Sensitivity X Phase1 Anxiety	-.30	.045

Note: $R^2 = .01$ for Step 1, $\Delta R^2 = .05$ for Step 2, $\Delta R^2 = .00$ for Step 3, $\Delta R^2 = .00$ for Step 4, $\Delta R^2 = .05$ for Step 5.

When Phase 1 depression was the index of prenatal stress, the overall model did not reach significance ($F(8,82) = 1.02$, $p > .05$). There was no main effect of maternal sensitivity on infant baseline cortisol levels when the influence of the covariates had been controlled for. Entering maternal sensitivity into step 4 of the model made no significant contribution and accounted for no further variance. Entering the interaction term (maternal sensitivity and Phase 1 depression) into step 5 had an effect on the model that approached significance and accounted for 4% of the variance. In a larger sample an effect of this magnitude may well reach significance. This finding indicates that the strength of the prediction from maternal prenatal depression to infant baseline cortisol is also moderated by maternal sensitivity (see Table 25).

Table 25 Summary statistics for regression analysis of maternal sensitivity and the interaction between maternal sensitivity and Phase 1 depression as a predictor of infant baseline cortisol

	Standardised β	P value
Step 1		
Pre SF time	.01	.94
Step 2		
Pre SF time	-.02	.87
Maternal Age	-.10	.40
Age finished education	.09	.41
Smoking	.15	.19
Birth weight	.13	.25
Step 3		
Pre SF time	-.02	.89
Maternal Age	-.11	.36
Age finished education	.09	.42
Smoking	.17	.17
Birth weight	.12	.27
Phase 1 Depression	-.05	.68
Step 4		
Pre SF time	-.02	.88
Maternal Age	-.10	.43
Age finished education	.09	.43
Smoking	.17	.18
Birth weight	.13	.28
Phase 1 Depression	-.05	.68
Sensitivity	-.01	.96
Step 5		
Pre SF time	.03	.79
Maternal Age	-.11	.38
Age finished education	.06	.58
Smoking	.13	.30
Birth weight	.10	.37

Phase 1 Depression	.20	.27
Sensitivity	.02	.87
Sensitivity X Phase1 Depression	-.32	.07

Note: $R^2 = .01$ for Step 1, $\Delta R^2 = .05$ for Step 2, $\Delta R^2 = .00$ for Step 3, $\Delta R^2 = .00$ for Step 4, $\Delta R^2 = .04$ for Step 5.

3.6.2 Associations between maternal sensitivity and infant cortisol reactivity, alone and in interaction with maternal stress at Phase 1

When Phase 1 anxiety was the index of prenatal stress, the overall model reached significance ($F(9,81) = 3.79$, $p < .001$). There was no main effect of maternal sensitivity on infant cortisol reactivity when the influence of the covariates had been controlled for. Entering maternal sensitivity into step 5 of the model made no significant contribution and accounted for 1% of variance. Entering the interaction term (maternal sensitivity and Phase 1 anxiety) into step 6 had no significant effect on the model and accounted for no further variance (see Table 26).

Table 26 Summary statistics for regression analysis of maternal sensitivity and the interaction between maternal sensitivity and Phase 1 anxiety as a predictor of infant cortisol reactivity

	Standardised β	P value
Step 1		
Pre SF time	-.21	.05
Step 2		
Pre SF time	-.21	.03
Baseline cortisol	-.46	.00
Step 3		
Pre SF time	-.21	.04
Baseline cortisol	-.47	.00
Maternal Age	-.12	.21
Age finished education	-.07	.46
Smoking	.06	.59
Birth weight	-.05	.58
Step 4		
Pre SF time	-.21	.04
Baseline cortisol	-.47	.00
Maternal Age	-.12	.22
Age finished education	-.07	.46
Smoking	.06	.50
Birth weight	-.06	.58
Phase 1 Anxiety	-.01	.91
Step 5		
Pre SF time	-.18	.06
Baseline cortisol	-.47	.00
Maternal Age	-.17	.13
Age finished education	-.09	.36
Smoking	.05	.60
Birth weight	-.07	.48
Phase 1 Anxiety	-.02	.85

Sensitivity	.10	.37
Step 6		
Pre SF time	-.19	.07
Baseline cortisol	-.47	.00
Maternal Age	-.17	.14
Age finished education	-.09	.37
Smoking	.06	.60
Birth weight	-.07	.49
Phase 1 Anxiety	-.02	.85
Sensitivity	.10	.37
Sensitivity X Phase1 Anxiety	.01	.95

Note: $R^2 = .04$ for Step 1, $\Delta R^2 = .21$ for Step 2, $\Delta R^2 = .04$ for Step 3, $\Delta R^2 = .00$ for Step 4, $\Delta R^2 = .01$ for Step 5, $\Delta R^2 = .00$ for Step 6

When Phase 1 depression was the index of prenatal stress, the overall model reached significance ($F(9,81) = 3.98$, $p < .001$). There was no main effect of maternal sensitivity on infant cortisol reactivity when the influence of the covariates had been controlled for. Entering maternal sensitivity into step 5 of the model made no significant contribution and accounted for 1% of variance. Entering the interaction term (maternal sensitivity and Phase 1 anxiety) into step 6 had no significant effect on the model and accounted for a further 1% of the variance. Maternal sensitivity does not play a moderating role in the association between Phase 1 depression and infant cortisol reactivity (see Table 27).

Table 27 Summary statistics for regression analysis of maternal sensitivity and the interaction between maternal sensitivity and Phase 1 depression as a predictor of infant cortisol reactivity

	Standardised β	P value
Step 1		
Pre SF time	-.21	.05
Step 2		
Pre SF time	-.21	.03
Baseline cortisol	-.46	.00
Step 3		
Pre SF time	-.21	.04
Baseline cortisol	-.47	.00
Maternal Age	-.12	.21
Age finished education	-.07	.46
Smoking	.06	.59
Birth weight	-.05	.58
Step 4		
Pre SF time	-.20	.03
Baseline cortisol	-.47	.00
Maternal Age	-.11	.26
Age finished education	-.07	.47
Smoking	.03	.76
Birth weight	-.05	.62
Phase 1 Depression	-.06	.56
Step 5		
Pre SF time	-.19	.05
Baseline cortisol	-.47	.00
Maternal Age	-.16	.17
Age finished education	-.09	.38
Smoking	.03	.80
Birth weight	-.07	.52
Phase 1 Depression	.07	.53

Sensitivity	.10	.36
Step 6		
Pre SF time	-.17	.10
Baseline cortisol	-.49	.00
Maternal Age	-.16	.15
Age finished education	-.10	.33
Smoking	.01	.91
Birth weight	-.07	.47
Phase 1 Depression	.18	.27
Sensitivity	.11	.31
Sensitivity X Phase1 Depression	-.14	.36

Note: $R^2 = .04$ for Step 1, $\Delta R^2 = .21$ for Step 2, $\Delta R^2 = .04$ for Step 3, $\Delta R^2 = .01$ for Step 4, $\Delta R^2 = .01$ for Step 5, $\Delta R^2 = .01$ for Step 6.

3.6.3 Associations between maternal sensitivity and infant baseline cortisol, alone and in interaction with maternal stress at Phase 2

When Phase 2 anxiety was the index of prenatal stress, the overall model failed to reach significance ($F(8,82) = .57$, $p > .05$). There was no main effect of maternal sensitivity on infant baseline cortisol when the influence of the covariates had been controlled for. Entering maternal sensitivity into step 4 of the model made no significant contribution and accounted for no additional variance. Entering the interaction term (maternal sensitivity and Phase 2 anxiety) into step 5 had no significant effect on the model and accounted for no additional variance. Maternal sensitivity does not play a moderating role in the association between Phase 2 anxiety and infant baseline cortisol (see Table 28).

Table 28 Summary statistics for regression analysis of maternal sensitivity and the interaction between maternal sensitivity and Phase 2 anxiety as a predictor of infant baseline cortisol

	Standardised β	P value
Step 1		
Pre SF time	.01	.94
Step 2		
Pre SF time	-.02	.87
Maternal Age	-.10	.40
Age finished education	.09	.41
Smoking	.15	.19
Birth weight	.13	.25
Step 3		
Pre SF time	-.02	.85
Maternal Age	-.09	.42
Age finished education	.09	.41
Smoking	.15	.19
Birth weight	.13	.25
Phase 2 Anxiety	.03	.81
Step 4		
Pre SF time	-.02	.85
Maternal Age	-.09	.47
Age finished education	.09	.42
Smoking	.15	.20
Birth weight	.13	.26
Phase 2 Anxiety	-.03	.81
Sensitivity	.00	.99
Step 5		
Pre SF time	-.02	.88
Maternal Age	-.10	.45
Age finished education	.09	.46
Smoking	.15	.21
Birth weight	.12	.29

Phase 2 Anxiety	.08	.62
Sensitivity	.01	.97
Sensitivity X Phase 2 Anxiety	-.08	.65

Note: $R^2 = .01$ for Step 1, $\Delta R^2 = .05$ for Step 2, $\Delta R^2 = .00$ for Step 3, $\Delta R^2 = .00$ for Step 4, $\Delta R^2 = .00$ for Step 5.

When Phase 2 depression was the index of prenatal stress, the overall model did not reach significance ($F(8,82) = .09$, $p > .05$). There was no main effect of maternal sensitivity on infant baseline cortisol levels when the influence of the covariates had been controlled for. Entering maternal sensitivity into step 4 of the model made no significant contribution and accounted for no further variance. Entering the interaction term (maternal sensitivity and Phase 1 depression) into step 5 had an effect on the model that was not significance however, it accounted for 3% of the variance (see Table 29).

Table 29 Summary statistics for regression analysis of maternal sensitivity and the interaction between maternal sensitivity and Phase 2 depression as a predictor of infant baseline cortisol

	Standardised β	P value
Step 1		
Pre SF time	.01	.94
Step 2		
Pre SF time	-.02	.87
Maternal Age	-.10	.40
Age finished education	.09	.41
Smoking	.15	.19
Birth weight	.13	.25
Step 3		
Pre SF time	-.02	.87
Maternal Age	-.10	.39
Age finished education	.09	.41
Smoking	.17	.19
Birth weight	.13	.24
Phase 2 Depression	-.03	.78
Step 4		
Pre SF time	-.02	.87
Maternal Age	-.10	.46
Age finished education	.09	.42
Smoking	.17	.19
Birth weight	.13	.25
Phase 2 Depression	-.03	.78
Sensitivity	-.00	.98
Step 5		
Pre SF time	-.02	.87
Maternal Age	-.16	.22
Age finished education	.02	.53
Smoking	.12	.35
Birth weight	.09	.44

Phase 2 Depression	.20	.28
Sensitivity	.04	.77
Sensitivity X Phase 2 Depression	-.30	.10

Note: $R^2 = .01$ for Step 1, $\Delta R^2 = .05$ for Step 2, $\Delta R^2 = .00$ for Step 3, $\Delta R^2 = .00$ for Step 4, $\Delta R^2 = .03$ for Step 5.

3.6.4 Associations between maternal sensitivity and infant cortisol reactivity, alone and in interaction with maternal stress at Phase 2

When Phase 2 anxiety was the index of prenatal stress, the overall model reached significance ($F(9,81) = 3.82$, $p < .001$). There was no main effect of maternal sensitivity on infant cortisol reactivity when the influence of the covariates had been controlled for. Entering maternal sensitivity into step 5 of the model made no significant contribution and accounted for 1% of variance. Entering the interaction term (maternal sensitivity and Phase 2 anxiety) into step 6 had no significant effect on the model and accounted for no further variance. Maternal sensitivity does not play a moderating role in the association between maternal anxiety at Phase 2 and infant cortisol reactivity (see Table 30).

Table 30 Summary statistics for regression analysis of maternal sensitivity and the interaction between maternal sensitivity and Phase 2 anxiety as a predictor of infant cortisol reactivity

	Standardised β	P value
Step 1		
Pre SF time	-.21	.05
Step 2		
Pre SF time	-.21	.03
Baseline cortisol	-.46	.00
Step 3		
Pre SF time	-.21	.04
Baseline cortisol	-.47	.00
Maternal Age	-.12	.22
Age finished education	-.07	.47
Smoking	.06	.59
Birth weight	-.05	.59
Step 4		
Pre SF time	-.21	.04
Baseline cortisol	-.47	.00
Maternal Age	-.12	.22
Age finished education	-.07	.47
Smoking	.06	.59
Birth weight	-.05	.59
Phase 2 Anxiety	-.02	.83
Step 5		
Pre SF time	-.19	.06
Baseline cortisol	-.47	.00
Maternal Age	-.17	.14
Age finished education	-.09	.38
Smoking	.05	.62
Birth weight	-.07	.48
Phase 2 Anxiety	-.03	.78

Sensitivity	.10	.37
Step 6		
Pre SF time	-.18	.06
Baseline cortisol	-.47	.00
Maternal Age	-.17	.13
Age finished education	-.10	.35
Smoking	.05	.64
Birth weight	-.06	.46
Phase 2 Anxiety	.07	.61
Sensitivity	.11	.35
Sensitivity X Phase2 Anxiety	-.06	.67

Note: $R^2 = .04$ for Step 1, $\Delta R^2 = .21$ for Step 2, $\Delta R^2 = .04$ for Step 3, $\Delta R^2 = .00$ for Step 4, $\Delta R^2 = .01$ for Step 5, $\Delta R^2 = .00$ for Step 6

When Phase 2 depression is the index of prenatal stress, the overall model reached significance ($F(9,81) = 3.81$, $p < .001$). There was no main effect of maternal sensitivity on infant cortisol reactivity when the influence of the covariates had been controlled for. Entering maternal sensitivity into step 5 of the model made no significant contribution and accounted for 1% of variance. Entering the interaction term (maternal sensitivity and Phase 2 depression) into step 6 had no significant effect on the model and accounted for a further 1% of the variance. Maternal sensitivity does not play a moderating role in the association between maternal depression at Phase 2 and infant cortisol reactivity (see Table 31).

Table 31 Summary statistics for regression analysis of maternal sensitivity and the interaction between maternal sensitivity and Phase 2 depression as a predictor of infant cortisol reactivity

	Standardised β	P value
Step 1		
Pre SF time	-.21	.05
Step 2		
Pre SF time	-.21	.03
Baseline cortisol	-.46	.00
Step 3		
Pre SF time	-.21	.04
Baseline cortisol	-.47	.00
Maternal Age	-.12	.21
Age finished education	-.07	.46
Smoking	.06	.59
Birth weight	-.05	.58
Step 4		
Pre SF time	-.21	.04
Baseline cortisol	-.47	.00
Maternal Age	-.13	.21
Age finished education	-.07	.46
Smoking	.06	.58
Birth weight	-.05	.59
Phase 2 Depression	-.01	.90
Step 5		
Pre SF time	-.19	.06
Baseline cortisol	-.47	.00
Maternal Age	-.17	.13
Age finished education	-.09	.37
Smoking	.06	.61
Birth weight	-.07	.49
Phase 2 Depression	-.01	.91

Sensitivity	.10	.38
Step 6		
Pre SF time	-.19	.06
Baseline cortisol	-.46	.00
Maternal Age	-.16	.19
Age finished education	-.09	.40
Smoking	.07	.56
Birth weight	-.06	.55
Phase 2 Depression	-.06	.70
Sensitivity	.09	.43
Sensitivity X Phase2 Depression	.07	.69

Note: $R^2 = .04$ for Step 1, $\Delta R^2 = .21$ for Step 2, $\Delta R^2 = .04$ for Step 3, $\Delta R^2 = .00$ for Step 4, $\Delta R^2 = .01$ for Step 5, $\Delta R^2 = .01$ for Step 5.

3.7 Controlling for concurrent (Phase 6) mood

Another regression analysis was run to examine whether the prediction from Phase 1 anxiety in interaction with maternal sensitivity to infant baseline cortisol remained after controlling for maternal anxiety symptoms at Phase 6. As the regression model containing the entire list of possible confounding variable was at the absolute limit of its statistical power and none of these variables had made a significant contribution to the model, a decision was made to remove all the control variables from the model. Infant baseline cortisol was the dependant variable. Then, by entering Phase 1 anxiety, maternal sensitivity and Phase 6 anxiety at the first step and the interaction term (Phase 1 anxiety by maternal sensitivity) at step 2, a regression model was constructed that had statistical power to examine any possible contribution of Phase 6 anxiety on the association found between Phase 1 anxiety and infant baseline cortisol in the presence of maternal sensitivity, results are summarised in Table 32.

Table 32 Summary statistics for regression analysis of the interaction between maternal sensitivity and Phase 1 anxiety as a predictor of infant baseline cortisol after controlling for Phase 6 anxiety

	Standardised β	P value
Step 1		
Sensitivity	-.03	.76
Phase 6 Anxiety	.03	.79
Phase 1 Anxiety	-.07	.52
Step 2		
Sensitivity	-.03	.76
Phase 6 Anxiety	.04	.74
Phase 1 Anxiety	.15	.30
Phase 1 Anxiety X Sensitivity	-.34	.02

Note: $R = .08$ for step 1, $\Delta R^2 = .07$ for Step 2

Overall the model was not significant ($F(4,86) = 1.63$, $p > .05$). Step 2 accounted for 7% of the variance and the only variable contributing significantly to the model was the interaction term (Phase 1 anxiety and maternal sensitivity). Therefore, the interaction between maternal sensitivity and Phase 1 anxiety as a predictor of infant baseline cortisol remained significant after controlling for the effects of concurrent maternal mood, evidenced by Phase 6 anxiety symptoms.

Given that the prediction from Phase 1 anxiety to infant baseline cortisol was only significant in the presence of maternal sensitivity, it is possible that any relationship between Phase 6 anxiety and infant cortisol would also be in interaction with sensitivity. Therefore, another regression analysis was run to control for any effect of Phase 6 anxiety in interaction with maternal sensitivity on infant baseline cortisol. Again, infant baseline cortisol was the dependant variable and step 1contained maternal sensitivity, Phase 1 anxiety and Phase 6 anxiety. One interaction term (Phase 6 anxiety and maternal sensitivity) was entered into step 2 and finally, the other interaction term (Phase 1 anxiety and maternal sensitivity) was entered to examine

whether it still predicted infant cortisol after controlling for the Phase 6 anxiety by sensitivity interaction. (Results are summarised in Table 33).

Table 33 Summary statistics for regression analysis of the interaction between maternal sensitivity and Phase 1 anxiety as a predictor of infant baseline cortisol after controlling for Phase 6 anxiety in interaction with maternal sensitivity

	Standardised β	P value
Step 1		
Sensitivity	-.03	.76
Phase 6 Anxiety	.03	.79
Phase 1 Anxiety	-.07	.52
Step 2		
Sensitivity	-.04	.75
Phase 6 Anxiety	.20	.21
Phase 1 Anxiety	-.08	.50
Phase 6 Anxiety X Sensitivity	-.23	.13
Step 3		
Sensitivity	-.33	.75
Phase 6 Anxiety	.13	.42
Phase 1 Anxiety	.12	.41
Phase 6 Anxiety X Sensitivity	-.13	.42
Phase 1 Anxiety X Sensitivity	-.30	.045

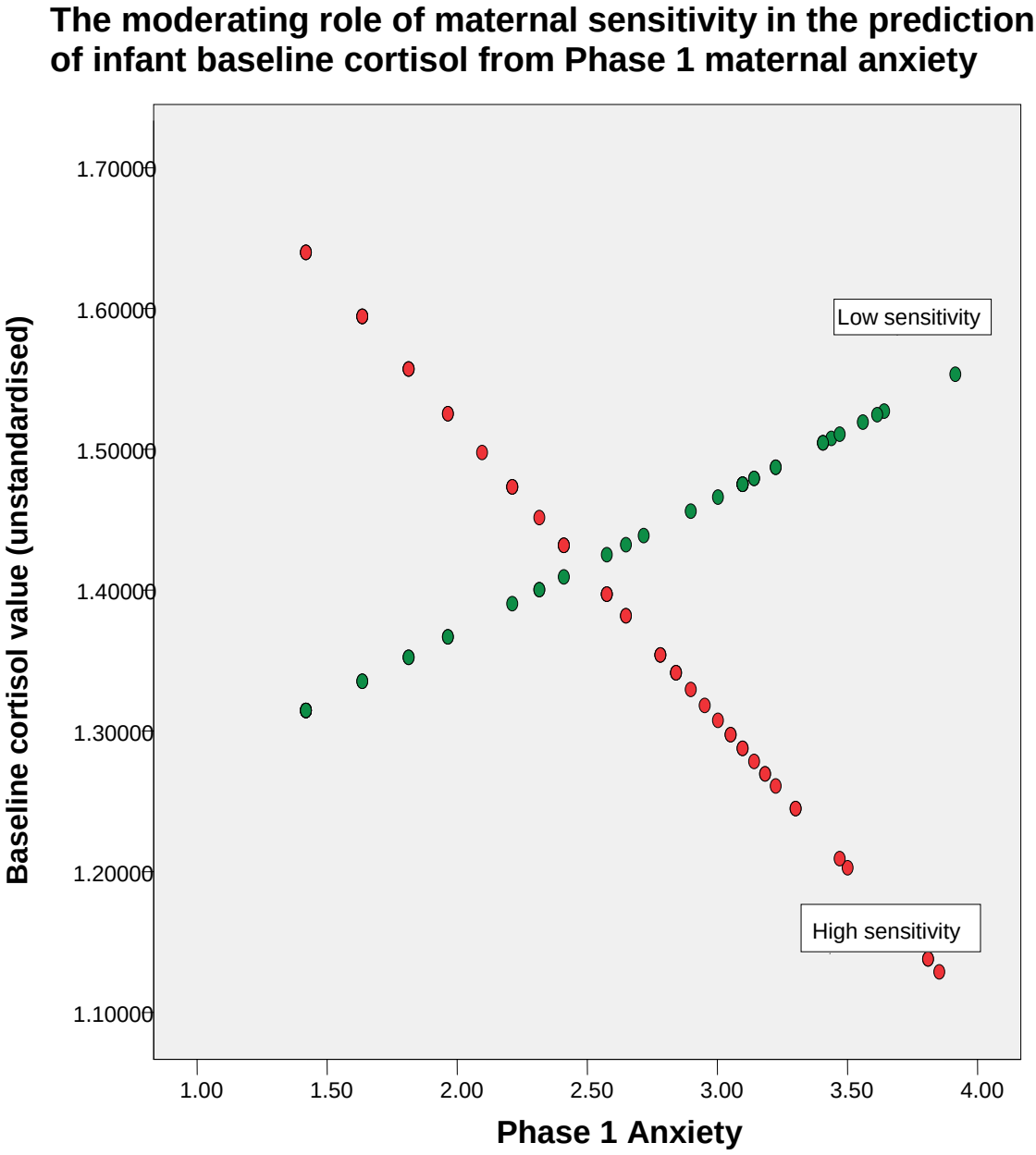
Note: $R = .08$ for step 1, $\Delta R^2 = .03$ for Step 2, $\Delta R^2 = .08$ for Step 3

Overall the model was not significant ($F(5,85) = 1.43$, $p > .05$). Step 2 accounted for 3% of the variance and none of the variables made a significant contribution to the model. The interaction between maternal sensitivity and Phase 1 anxiety as a predictor of infant baseline cortisol remained significant after controlling for the effects of concurrent maternal mood, evidenced by the interaction between Phase 6 anxiety symptoms and maternal sensitivity.

3.8 Graphical representation of the moderating role of maternal sensitivity in the prediction of infant baseline cortisol from Phase 1 maternal anxiety

Figure 5 depicts the relationship between Phase 1 maternal anxiety and infant baseline cortisol for the low and high maternal sensitivity groups. As predicted, for infants of mothers in the low sensitivity group, increased exposure to maternal anxiety during the 2nd trimester of pregnancy was predictive of increased baseline cortisol levels. However, instead of high levels of maternal sensitivity simply buffering the effect of foetal exposure to maternal anxiety during the 2nd trimester of pregnancy on subsequent infant cortisol levels, there is a relationship in the opposite direction to that seen within the low sensitivity group. In the presence of highly sensitive maternal caregiving, increased exposure to maternal anxiety during the 2nd trimester of pregnancy is associated with decreased baseline cortisol levels in 6 month old infants.

Figure 5 Graphical representation of the moderating role of maternal sensitivity in the prediction of infant baseline cortisol from Phase 1 maternal anxiety



4. Discussion

The primary aim of the current study was to test a priori hypotheses pertaining to the relationship between indices of maternal prenatal stress, namely anxiety and depression symptoms, and infant cortisol levels, both at baseline and in response to a social stressor paradigm administered at 6 months of age, after potential confounding variables had been controlled for. Secondly, the study aimed to examine whether the timing of prenatal stress during pregnancy, 2nd trimester versus 3rd trimester, influenced the infant outcome. The final aim was to examine whether maternal sensitive behaviour towards her infant at 6 months played a moderating role in the association between maternal prenatal anxiety/depression and infant cortisol levels.

The indices of stress used in this study were scores on self-report anxiety and depression symptom scales. Conceptualising stress in this way broadly reflects Lazarus and Folkman's (1984) view of stress as a multidimensional construct involving a transaction between person and environment. Although using symptom scales does not capture stress provoking factors, measuring stress as emotional output means that the result of individual differences in appraisal and coping are intrinsically captured within the index of distress. The use of symptomatic measures of stress as predictor variables and both baseline cortisol and cortisol reactivity as outcome measures is also in line with much of the literature investigating relationships between prenatal stress and infant HPA axis function to date (Van den Bergh et al., 2008; Field et al., 2004; O'Connor et al., 2005).

The results of the current study will now be summarised and comparisons will be drawn with findings from previous research. A discussion of the study's strengths and limitations including the methodological challenges encountered will follow. Finally recommendations for future research and the clinical implications of the findings will be considered.

4.1 Overview of study results

Contrary to prediction, there was no main effect of maternal prenatal anxiety or depression on infant cortisol outcome. Neither symptoms of anxiety or depression

measured in the 2nd trimester nor the 3rd trimester of pregnancy were significantly associated with infant baseline cortisol level or cortisol reactivity. There was also no main effect of sensitivity on infant cortisol levels. However, there was a significant association between maternal anxiety at Phase 1 (2nd trimester of pregnancy) and infant baseline cortisol in interaction with maternal sensitivity, indicating that infant cortisol levels varied by maternal sensitivity and prenatal anxiety. In the presence of low sensitivity, higher levels of prenatal maternal anxiety were associated with higher infant baseline cortisol values. However, in the presence of high sensitivity, infant baseline cortisol increased as maternal prenatal anxiety decreased. In some respects, this finding supports the hypothesis that high maternal sensitivity attenuates the effect of maternal prenatal anxiety on infant cortisol but at the same time it reveals a group of infants of highly sensitive mothers with low anxiety during pregnancy who have high cortisol levels which was not expected. Results for maternal depression at Phase 1 were similar, there was no significant association between maternal sensitivity and infant cortisol but the sensitivity by maternal depression interaction approached significance ($p = .07$), indicating a trend towards higher baseline cortisol levels in infants of prenatally depressed mothers in the presence of low maternal sensitivity. There was no association between maternal mood at Phase 2 (3rd trimester of pregnancy) and infant baseline cortisol or cortisol reactivity, alone or in interaction with sensitivity. The findings from this investigation will now each be discussed in more detail in the context of previous work in the area.

4.2 Hypothesis 1: Is prenatal maternal anxiety or depression associated with increased infant basal cortisol levels and cortisol reactivity to a social stressor after controlling for possible demographic confounds (maternal age, education and deprivation), smoking behaviour in pregnancy, birth weight and timing of cortisol sampling?

This study found no significant linear associations between prenatal maternal stress, as indexed by anxiety and depression symptoms, on infant baseline cortisol levels or cortisol reactivity. The a priori hypothesis was not supported at either Phase 1 or Phase 2 of the study. This finding is contrary to what had been predicted on the basis of animal studies (Weinstock, 2005). However given the limitations of the human literature, reviewed in Section 1.7, it is not possible to conclude whether or not these

findings differ from those of previous work. Four previous studies have examined the relationship between prenatal stress and infant cortisol, but only two of these (Grant et al., 2009; Kaplan et al., 2008) were prospective and directly comparable to the work within this thesis. The study by Grant et al. (2009) was similar in that it included prenatal measurement of depression and anxiety and an assessment of cortisol reactivity following the still-face social stress paradigm and they reported a significant interaction between prenatal anxiety diagnosis and greater infant cortisol response to the still-face. However their study yielded complex results in which the prenatally anxious group displayed decreasing cortisol levels from baseline to 25 minutes post stress followed by an increase in cortisol thereafter. The effect of prenatal anxiety was accounted for by a difference between the anxiety diagnosis and control groups in the cortisol slope between the 25 and 40 minutes post-test samples. Given that there was no difference between the groups' cortisol levels at any other time point, the authors speculated that there was a possibility that this was a chance finding within their small sample. The results of the current study support this interpretation and are also consistent with Kaplan et al. (2008) who found no main effect of prenatal diagnosis of mood or anxiety disorder in predicting infant baseline cortisol levels in their prospective study. However, it must be noted that the sample included in their study was only 33 women and just 13 reached diagnostic criteria for mood or anxiety disorder. These results will therefore be vulnerable to the influence of a few large values and the numbers are too small to draw reliable conclusions from. Finally, Brennan et al. (2008) found that a lifetime history of maternal depression was associated with increased baseline cortisol in 6 month old infants but this was based on retrospective reporting of mood disorders. The association was between lifetime history of depression and infant cortisol rather than the effect of exposure to maternal stress in utero which is the focus of the current study. One might also argue that their results may reflect underlying genetic influences on infant outcomes.

In terms of cortisol reactivity, the findings of the present study are not consistent with the well described association between maternal prenatal stress and offspring HPA axis reactivity in the animal literature (Henry et al., 1994; Weinstock et al., 1992; Barbazanges et al., 1996). Within the human literature, the association between prenatal maternal stress and infant cortisol reactivity has not been firmly established. Leung et al.'s (2010) findings were not supported by the present study. They reported

that perceived maternal stress during pregnancy predicted infant cortisol reactivity at 10 months. However, their 10 month sample consisted of only 26 infants. In a sample this small, just one or two large values could affect the results greatly and therefore cannot be relied upon as firm evidence of the association described. The use of parametric testing without mention of the distributions of the data and the retrospective report of maternal stress are further reasons why the results should be interpreted with caution. Nor did the findings of the present thesis support Brennan et al.'s (2008) reported association between peripartum depression (when comorbid with anxiety) and infant cortisol reactivity at 6 months. In summary, there are no other studies that have examined the questions raised in this thesis with adequate sample size that have provided conclusive results. Thus current knowledge is too limited to be able to judge whether or not the current findings are consistent with previous work.

There are a number of possible explanations for the differences between findings in previous studies and the study reported here. The indices of maternal stress used are not always comparable. Some studies (Grant et al., 2009; Brennan et al., 2008; Kaplan et al., 2008) use a diagnosis of depression or anxiety as the index of maternal prenatal stress and compare the diagnosed groups with normal controls on infant cortisol outcome rather than examining the relationship between scores on a symptom scale and infant outcome as the present study has done. Direct comparison between studies that use symptom scales and studies that use diagnoses as the predictor variable may be difficult as they might be capturing different aspects of maternal stress in terms of severity, chronicity and underlying psychology and physiology. Sample characteristics also vary across studies (e.g. low risk community samples versus higher psychosocial risk samples such as the present sample) making cross-study comparability and interpretation of results difficult. Different stressor paradigms have been used across the literature which could be another explanation for the inconsistencies in the findings. Grant et al. (2009) used a modified version of the still-face procedure which included a 1 minute period in which the mother left the room in order to make the procedure more likely to elicit distress in the infant. Approximately half of their sample responded to the modified procedure with a significant increase in cortisol. The present study used the standard still-face procedure which is a well established and widely used paradigm in the study of stress in infancy. However, it may have been a slightly milder stressor than Grant et al.'s (2009) version as it did not

involve total separation from the mother so it is possible that the paradigm used was not stressful enough to produce an adequate cortisol response. A stronger stressor may have been able to achieve more consistent results. Leung et al. (2010) used a toy removal procedure designed to elicit frustration in the infant. The procedure had similarities to the still-face as the mother was instructed to use the toy to play with their infants during the pre-frustration period and to assume a neutral expression and refrain from interacting with their infant during the 2 minute toy removal period and finally resume normal interaction with their infant. Nevertheless, the additional frustration element of the toy removal procedure may have made it a more powerful stressor paradigm which could partly explain the positive findings in relation to cortisol reactivity in Leung et al.'s (2010) study and makes cross-study comparison more difficult.

4.3 Hypothesis 2: Maternal sensitive behaviour towards her infant at 6 months will moderate the association between maternal prenatal anxiety/depression and infant cortisol levels. High levels of maternal sensitivity will buffer the effect of prenatal anxiety/depression on infant cortisol levels.

The present study found that infant physiology was significantly influenced by maternal caregiving. This is consistent with the animal literature (Francis & Diorio, 1999; Liu et al., 1997; Meaney, 2001) that has shown differences in maternal caregiving behaviours in rat dams to be associated with physiological markers of stress in their infants. As predicted, the sensitivity of maternal caregiving, measured during a playful mother-infant interaction at 6 months of age, moderated the effect of prenatal anxiety symptoms during the 2nd trimester of pregnancy (Phase 1) on infant baseline cortisol levels. In the low maternal sensitivity group, higher levels of anxiety at Phase 1 predicted higher infant baseline cortisol levels. The findings of this thesis are consistent with Kaplan et al. (2008) who also found that maternal sensitivity moderated the association between prenatal maternal anxiety and infant baseline cortisol. Infants in the clinically anxious group had significantly higher cortisol levels if they received low sensitive parenting but not if they received high sensitive parenting. Infants in their non anxious control group had low cortisol levels regardless of whether their mothers were rated high or low on sensitivity.

In some respects, the moderating role that maternal sensitivity plays in the association between prenatal stress and infant cortisol was found to be in the predicted direction in the current thesis. In the presence of low sensitivity, higher prenatal maternal anxiety predicts higher infant cortisol. So on the one hand the presence of higher maternal sensitivity appears to moderate the effect of prenatal anxiety on infant cortisol, suggesting that a high quality postnatal caregiving environment can reverse any possible epigenetic or programming routes to the association between prenatal stress and infant HPA axis function. However, on the other hand, the present study also revealed a surprising finding, the explanation for which is unclear. In the presence of highly sensitive maternal caregiving, decreased maternal anxiety during the 2nd trimester of pregnancy was associated with increased baseline cortisol levels in their 6 month old infants. So, instead of high levels of maternal sensitivity simply buffering the effect of foetal exposure to maternal anxiety during the 2nd trimester of pregnancy on subsequent infant cortisol levels, there was also a subgroup for whom the relationship was in the opposite direction to the low sensitivity group. Grant et al.'s (2009) findings in relation to maternal sensitivity are different again. They did not find maternal sensitivity moderated the interaction between prenatal anxiety and infant cortisol. They found that infant cortisol reactivity differed as a function of maternal sensitivity independently of prenatal anxiety diagnosis. Infants of highly sensitive mothers showed little change in cortisol levels in response to the still-face procedure, whereas infants of low sensitive mothers showed a significant decrease from baseline to 15 minutes post test and then an increase from 15 to 25 minutes post test. Again, this is not a simple relationship. The findings in the highly sensitive group support the theory that sensitive maternal behaviour can buffer the infants' HPA response to the stressor (Gunnar & Donzella, 2002; Calkins, 1994). However, in the low sensitive group it is unclear why the infants initially display a decrease in cortisol in response to the stressor and in fact the overall direction of the change is a decrease from baseline levels. Interestingly, a trend was noted for infants of low sensitive mothers to arrive in the lab with higher baseline levels. This raises the question of whether 'baseline' cortisol samples in laboratory-based stress studies represent a true baseline value or are reflecting a response to arriving in the laboratory and interacting with strangers, a novel and potentially stressful experience. It could be that infants in the low sensitivity group have a large response to arrival in the lab due to the lack of availability of the mother as an external regulator so the 'baseline' value actually

represents an already activated stress response, making the response to the laboratory stressor hard to interpret. If the baseline values were elevated in response to arrival at the laboratory, it would be harder for the still-face procedure to elicit a further increase in cortisol (see section 1.7.5) and this may partly explain why the findings in relation to cortisol reactivity were not significant in the present study. A study design that involved collection of baseline samples by the mother at home might be a way of resolving this issue.

In studies such as the present thesis, where the association between maternal prenatal stress and infant cortisol reactivity is under examination, it is likely that there may be subgroups of infants displaying different, possibly even opposite coritsol responses to the stressor paradigm. If for example, infants who experienced high levels of stress in utero react in the opposite direction to infants who were exposed to low levels of stress, the effects could cancel each other out, masking any significant bivariate associations. For this reason, it is important to try and identify these subgroups of infants in order to examine varying patterns of cortisol response. The present study identified subgroups based on the sensitivity of maternal responding within a play based parent-infant interaction and it was only when maternal prenatal stress was examined in interaction with sensitivity grouping that the significant association with infant baseline cortisol was revealed. It is possible that other sources of individual differences such as infant sex or behavioural and temperamental differences could relate to varying patterns of HPA reactivity and these other potential moderating factors warrant further investigation. The current findings have been interpreted in line with the view that the postnatal environment, specifically maternal caregiving behaviour, can influence infant biobehavioural development. However, as Hane and Fox, (2006) discuss, there is evidence that maternal behaviour and infant temperament interact reciprocally to influence developmental outcomes (Calkins, 2002). In the same way that maternal caregiving may shape infants' stress reactivity systems; infants' temperament may evoke differing maternal caregiving behaviours. Thus infants who are highly temperamentally 'difficult' may be more likely to receive insensitive parenting and this insensitive parenting may lead to epigenetic changes that effect the way the infant responds to stressors. Future studies may benefit from examining individual temperamental differences in infants in relation to the caregiving they receive and its impact on their physiological response to stress,

although Kaplan et al. (2008) and Hane and Fox (2006) both found that maternal sensitivity was not significantly associated with either subjective report or objective classification of infant temperament in their work. Longitudinal investigation of how these relationships change over time may also be informative.

In summary, the findings of the present study are consistent with the animal literature that suggests that maternal postnatal behaviour can play an important role in shaping offspring's HPA axis function and in modifying the impact of any potential prenatal programming effects of maternal stress on offspring stress physiology. Few studies test these relationships prospectively within humans and this study adds to a growing literature in the field.

4.4 Findings in relation to timing

The literature is divided as to the importance of timing of prenatal stress in relation to infant cortisol outcome. The present study measured anxiety and depression symptoms at 20 weeks of pregnancy (Phase 1) and again at 32 weeks gestation (Phase 2) in order to examine the possibility of there being a particular period of development when the foetus is most vulnerable to the effects of exposure to prenatal stress. Only exposure to higher prenatal maternal anxiety at Phase 1 in the presence of later low sensitivity was significantly associated with higher infant baseline cortisol levels. This is consistent with Van den Bergh et al. (2008) who found prenatal anxiety at 12-22 weeks to be significantly associated with diurnal cortisol profiles in both sexes and depressive symptoms in adolescent girls. No such associations were found for maternal anxiety at 23-32 or 32-40 weeks gestation in their study. In previous studies these authors found the effects of prenatal maternal anxiety on childhood behavioural disorders at 8-9 years and on cognitive functioning at 14-15 years old were confined to maternal anxiety at 12-22 weeks gestation (Van den Bergh et al., 2004, 2006b). Huizink et al. (2008) found that maternal prenatal exposure to stress during the 2nd trimester of pregnancy was associated with raised cortisol levels in their 14 year old offspring. This association was not present when the exposure to stress occurred during the 1st or 3rd trimesters. These studies suggest that the 2nd trimester pregnancy may be a critical time in the development of biological systems involved in the infants stress response and exposure to maternal anxiety during this period may

program the functioning of these systems. In contrast to these reports, O'Connor et al (2005) found that the impact of prenatal anxiety on the infant's HPA axis was strongest when the exposure occurred at 32 weeks gestation in a sample of 74 10 year olds. Overall these mixed results suggest further investigation into the importance of the timing of prenatal maternal stress on infant cortisol outcomes is required before firm conclusions can be drawn.

4.5 Strengths and limitations

4.5.1 Measures

This study adds to the literature by examining the effect of prenatal maternal stress on infant HPA axis function, alone and in interaction with maternal sensitivity in the largest prospective study of its kind to date. In addition, the effect of timing of prenatal stress was examined in order to contribute to a currently divided literature by measuring anxiety and depression symptoms at two time points during pregnancy. In addition to this, maternal anxiety and depression was also measured postnatally at the 6 month laboratory assessment in order to control for the effect of concurrent mood on infant outcome, and the significant findings remained after doing so. The measures used were all well established within the development literature to aid cross study comparison.

Maternal sensitivity was measured using the same rating scale as used by the largest child developmental research network, the NICHD, again for ease of comparability. Inter-rater reliability for the maternal sensitivity scale was high, so measurement error is unlikely to be a limitation. The NICHD sensitivity rating scale requires the observation of the mother and infant in a 15 minute playful interaction. This assessment of maternal sensitivity was independent of the stressor paradigm, providing a cross situational rating of parenting, rather than just a reflection of how the mother behaved in one specific interaction. However, the drawback of this method is that it does not show how the mother responded during the period in which the cortisol was being measured, so the effect of maternal behaviour within the stressor paradigm on subsequent infant cortisol is not known. It is possible that insensitive maternal behaviours could be stressful to the infant and have a direct impact on their

cortisol levels during the still-face procedure. However, using a stress paradigm that is a social interaction with the mother necessarily makes it hard to separate the source of stress from the caregiving quality. The fact that maternal sensitivity outside of the still-face procedure moderates the link between prenatal maternal anxiety and infant cortisol possibly suggests that the moderating effect is due to maternal behaviours shaping the structure and function of the HPA axis over some period of time rather than being limited to the cause of the current stress. In order to best establish the role of distal and proximal caregiving behaviours, a prospective design would be needed to examine sensitivity prior to the infant outcome as well as within the social stress paradigm. A further methodological issue that requires attention in future work relates to any assumption of the equivalence of the social stress paradigm across different infants. This may not necessarily be true, for example, the disengagement period could potentially be a relief rather than a stressor for infants of highly intrusive mothers. This is a potential limitation generalisable to all studies examining infant response to a social stressor via withdrawal of maternal interaction.

Another possible limitation of the current study may be the use of anxiety and depression symptom scales rather that diagnostic interviews. The use of symptom scales facilitated assessment of maternal mood at three different time points and conducting repeated diagnostic interviews would have been a strain on the available resources and on participant compliance within a longitudinal design. Also, even in a high risk sample there would be few women meeting diagnostic criteria, thereby reducing the statistical power to detect significant differences within the sample size available.

4.5.2 Cortisol

The methodological challenges of using salivary cortisol as an index of HPA axis function in infants are well documented in the literature (see Eglison et al., 2007 for a review) and have been discussed in this thesis (see section 1.8.1). Laboratory baseline cortisol levels are known to potentially be influenced by meals (de Weerth et al., 2003), sleeping (de Weerth & van Geert, 2002) and the use of cortisol based creams. In order to minimise the potential confounding effects of these factors, careful steps were taken to ensure that none of the samples were taken within 30 minutes of

sleeping or feeding. None of the present sample was using cortisol based creams. The time of day at which the sample was taken is another source of potential variation in cortisol values. Although no firm conclusions have been drawn regarding the age that the adult pattern of cortisol secretion (highest in the early morning and lowest at midnight) is established, several studies suggest that the circadian rhythm is in place by 2-3 months of age (Egliston et al., 2006). As it was not possible to see every dyad at the same time during the day, time of sampling was carefully noted in every case and used in statistical exploration. Bivariate analyses revealed specific associations between the time of baseline cortisol sampling and post social stressor levels of cortisol. Time of baseline assessment was therefore entered into multivariate analyses as a control variable as a precautionary step.

Another frequently discussed issue in the literature pertaining to infant HPA reactivity is that of timing of peak response to a stressor. The general consensus has been that peak cortisol levels were reached around 20 minutes after the beginning of the stressor (e.g. Gunnar et al., 1988; Lewis et al., 1993; Lewis & Ramsay, 1995) and the present study followed these authors. More recently, evidence (Ramsay & Lewis, 2003) has revealed that there may be greater variation between individuals in time taken to reach peak cortisol, with considerable number of infants reaching peak levels at 15 and 25 minutes as well as 20 minute post-stressor. Goldberg et al., (2003) actually found that as many 12-18 month year old reached peak cortisol 40 minutes post-stressor as did peak at 20 minutes. This emerging evidence suggests taking multiple post-stress samples beyond the usual 20 minutes would be advisable to sensitively measure cortisol reactivity and the present study is limited by its use of a single post stress sample which may have failed to capture individual difference in peak response.

Another challenge facing researchers measuring salivary cortisol in infants is collecting the sample. Some studies have reported high rates of data loss due to some infants becoming distressed during the collection procedure or not giving sufficient volumes for assay, which raises the problem of collection bias. It is possible that the most behaviourally reactive infants, in whom we would expect greater cortisol reactivity, may have been more likely to refuse saliva collection leading to type 2 error in the analysis. However, cortisol data loss in the WCHADS intensive sample was small. Out of the whole intensive sample (n = 276), 88.0% of infants gave both

samples. It is therefore unlikely that collection bias in the current study could have influenced the findings to any large degree.

4.5.3 Statistical analysis, power and sample size

The current study reported data from a sub-sample of the WCHADS intensive sample. The sample was limited in practical terms by the number of cases for which salivary cortisol samples had been processed by the laboratory and for which sensitivity had been rated at the time of writing. The a priori power calculation specified that 103 participants would have 80% power to detect a medium effect size with seven predictors in a regression model. Since full data was only available for 91 mother-infant dyads and multivariate analyses included up to a maximum of 9 variables, it is possible that certain regression analyses may have been somewhat underpowered. When data is available for the full WCHADS is available, it may be that smaller effects that were not detected in this sub-sample will become apparent. Some trends that were apparent in the current analysis such as the association between depression at Phase 1 in interaction with maternal sensitivity and infant baseline cortisol, may meet the criteria for statistical significance in a larger sample size.

A further limitation of the present study was the lack of correction for multiple comparisons within the bivariate analyses. Multiple comparisons increase the possibility that significant associations could be detected by chance giving rise to Type 1 errors. However, all the correlations found were in the expected direction so it is unlikely that they could be explained by chance alone. Following Rothman (1990) the significance level was kept at alpha $<.05$ in order to decrease the chances of Type 2 errors (rejecting a true significant result) which is more likely to occur if the significance level is adjusted to be more conservative. This approach was deemed appropriate as it would be unwise to prematurely reject significant findings at this early stage of investigation in this area of research, although it is acknowledged it does increase the possibility of Type 1 errors. Analyses on the entire WCHADS intensive sample when full data is available will clarify and confirm the reliability of the present findings.

4.2.4 Sample characteristics

A strength of this study was the systematic identification of the sample under investigation. Being a consecutive sample it is more likely to be free from referral bias and representative of the general population of first time mothers in the local geographical area. There was a high level of retention of participants across the phases of study and a high number (68.4%) of women eligible to take part in the 'extensive' study consented to do so. Subsequent to this, 61.6% of those approached to take part in the 'intensive' part of the study also agreed to do so. The sub-sample reported on in this thesis was a convenience sample from the WCHADS intensive sample and there was no known systematic bias in its selection as it was determined by the availability of data. The intensive study sample was stratified by relationship abuse and as such represents a higher risk community sample compared to other previous studies in the field. Comparisons made between the current sample and the rest of the intensive WCHADS sample revealed no difference in terms of maternal demographic characteristics indicating that the study sample was likely to be representative of the larger WCHADS intensive sample.

4.2.5 Other potential confounders

The rigorous controlling for the effects of potential confounding variables strengthens the conclusions that can be drawn from this study. Following previous findings, birth weight (Field et al., 2006; Rondo et al., 2003) smoking during pregnancy (Schuetze et al., 2008) were entered into the regression analyses along with maternal demographic variables that were significantly associated with any of the predictors variables. Time of day of cortisol sampling and concurrent maternal mood are also important covariates that were controlled for in these analyses in order to ensure that any associations found were directly due to prenatal stress and not other related factors. There is evidence in the literature that there may be other potential confounding factors that were not controlled for in the present study. Stressful delivery has been shown to be associated with cortisol response to stress in infants (Miller et al., 2005; Taylor et al., 2000; Brennan et al., 2008). However, with the current sample size of 91, there was a limit to the number of predictors that can be entered into the regression analyses and had any more been entered into the models, they would not have had sufficient power to detect any significant associations. It could be argued

that too many potential confounders were examined in the regression analyses reported in the current thesis. Most did not in fact make a significant contribution and perhaps could have been left out. However it was decided that to err on the side of caution was the best approach and they were included in the analyses.

4.6 Future studies

The field is in its infancy with only a handful of human studies published to date on the effect of maternal prenatal stress on subsequent infant HPA axis function. In light of the limitations and methodological issues discussed above further work and replications of previous studies is needed with a view to addressing these limitations wherever possible. However, ethical constraints within human research on stress limit the extent to which potential mechanisms and causation can be established.

Given that the present study revealed some surprising findings in relation to the moderating role of maternal sensitivity on the association between prenatal maternal anxiety and infant baseline cortisol level, namely that in the presence of high sensitivity, infant baseline cortisol levels increased as maternal prenatal anxiety decreased, the findings warrant replication with a larger sample size. When the data for the full WCHADS intensive sample is available, the regression analyses can be repeated and will have greater power to detect smaller effects and a more complete view of the relationships and possible multiple mechanisms can be established.

The interpretation of results involving examination of cortisol levels should take into consideration that the release of cortisol in the face of a stressor is an adaptive response in humans. For this reason, it is difficult to establish what is an adaptive response in the infant to the novel and potentially stressful experience of the laboratory visit and what is an elevated or maladaptive response that may infer vulnerability. It is possible that the group of infants with highly sensitive mothers who experience low levels of anxiety in pregnancy but then showed increased baseline cortisol levels at 6 months may have been displaying a prompt and strong but ultimately adaptive response to the stress following arrival at the laboratory that was appropriate for their stage of development, rather than a maladaptive response. In the presence of continued sensitive parental responsiveness it is likely that that by around

1 year of age these same infants may no longer display an increase in cortisol to a novel environment or to a social stressor (Gunnar et al., 2009). However, this remains to be tested. There is evidence to suggest that only insecurely attached children (Spangler & Grossmann, 1993) or insecurely attached children who were also highly fearful (Gunnar et al., 1996b) demonstrate an increase in cortisol to maternal separation at 1 year of age. These insecurely attached infants may have experienced less sensitive maternal care in the preceding first year of life and this environment may have led to changes in the organization of stress regulation mechanisms in the infant, such as the HPA axis, which persist and effect how they respond to future stressors. This is referred to by evolutionary biologists as neural plasticity (Hane & Fox, 2006). Thus, future studies need to address later outcomes in the infants in the high sensitivity/low anxiety group relative to those in the low sensitivity/ high anxiety groups. The present study found them to have similar baseline cortisol level a 6 months of age, but they may well be on very different developmental trajectories and long term follow up of these subgroups may reveal important differences in HPA axis functioning over time. Future studies addressing the behavioural outcomes in these apparently physiologically dysregulated infants are needed to gauge the consequences of dysregulation within biological stress systems on childhood social and emotional wellbeing.

4.7 Clinical implications

The prevalence of women experiencing anxiety and depression symptoms during pregnancy has been found to be as high as 54% and 37% respectively (Lee, Lam, Sze Mun Lau, Chong, Chui & Fong, 2007). It is well established that the experience of these symptoms is common and can be highly distressing problems for pregnant women and associated with negative behavioural and emotional outcomes in their offspring. Results from the Avon Longitudinal Study of Parents and Children (ALSPAC) showed that children whose mothers experienced high levels of anxiety in late pregnancy exhibited higher rates of behavioural/emotional problems at 81 months of age after controlling for obstetric risks, psychosocial disadvantage, and postnatal anxiety and depression (O'Connor et al., 2003). From the literature, it is apparent that reducing maternal anxiety during pregnancy using preventative interventions is warranted in the hope of diminishing the chance of associated negative outcomes in

their offspring. The present study found continuity in maternal mood when measured during the 2nd and 3rd trimesters of pregnancy and 6 months postnatally, adding to the rationale for prenatal interventions and early identification of these women. Higher maternal anxiety during the 2nd trimester specifically, followed by a caregiving environment characterized by low maternal sensitivity, was be particularly influential in the subsequent function of the infant HPA axis in the current study, suggesting that interventions timed during or prior to this stage of pregnancy might be optimally effective. Findings from this study further indicate that interventions aimed at enhancing maternal sensitivity at the behavioural level may buffer these direct associations between mood and the developing HPA axis. Young maternal age was found to be strongly associated with lower sensitivity in this primiparous sample. Such findings suggest targeting interventions to particular subgroups who are more likely to have lowered sensitivity may be helpful. Research into the development of interventions that focus on enhancing sensitive maternal behaviour is underway. Bakermans-Kranenburg, van Ijzendoorn and Juffer (2003) reported a meta-analysis in which such interventions were found to be significantly and moderately effective in enhancing maternal sensitivity ($d = 0.33$, $p < .001$) and that sensitivity interventions with large effect sizes were also successful in enhancing infant attachment security. However, whether early interventions designed to increase maternal sensitivity could be effective in preventing less optimal outcomes associated with physiological and emotional dysregulation is yet to be investigated.

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4.8 Conclusion

There is a considerable body of evidence in the animal and human literature linking maternal prenatal stress with adverse neurodevelopmental and behavioural outcomes in offspring. The mechanisms behind these associations are still unknown. The present study found no main effect of maternal prenatal anxiety or depression on infant HPA axis function but a significant relationship was observed in the context of higher maternal sensitivity. This study adds to the literature on the timing of prenatal stress and suggests that exposure during the 2nd trimester of pregnancy, rather than the last trimester, may be important. The quality of the postnatal caregiving environment appears to buffer any prenatal programming effect of maternal stress on the function of the infant HPA axis. It may be that sensitive maternal behaviour shapes the

development of biobehavioural systems that influence the infant's capacity for successful self-regulation, without which the individual may be rendered temperamentally vulnerable and at increased the risk of developing behavioural and emotional problems in later childhood.

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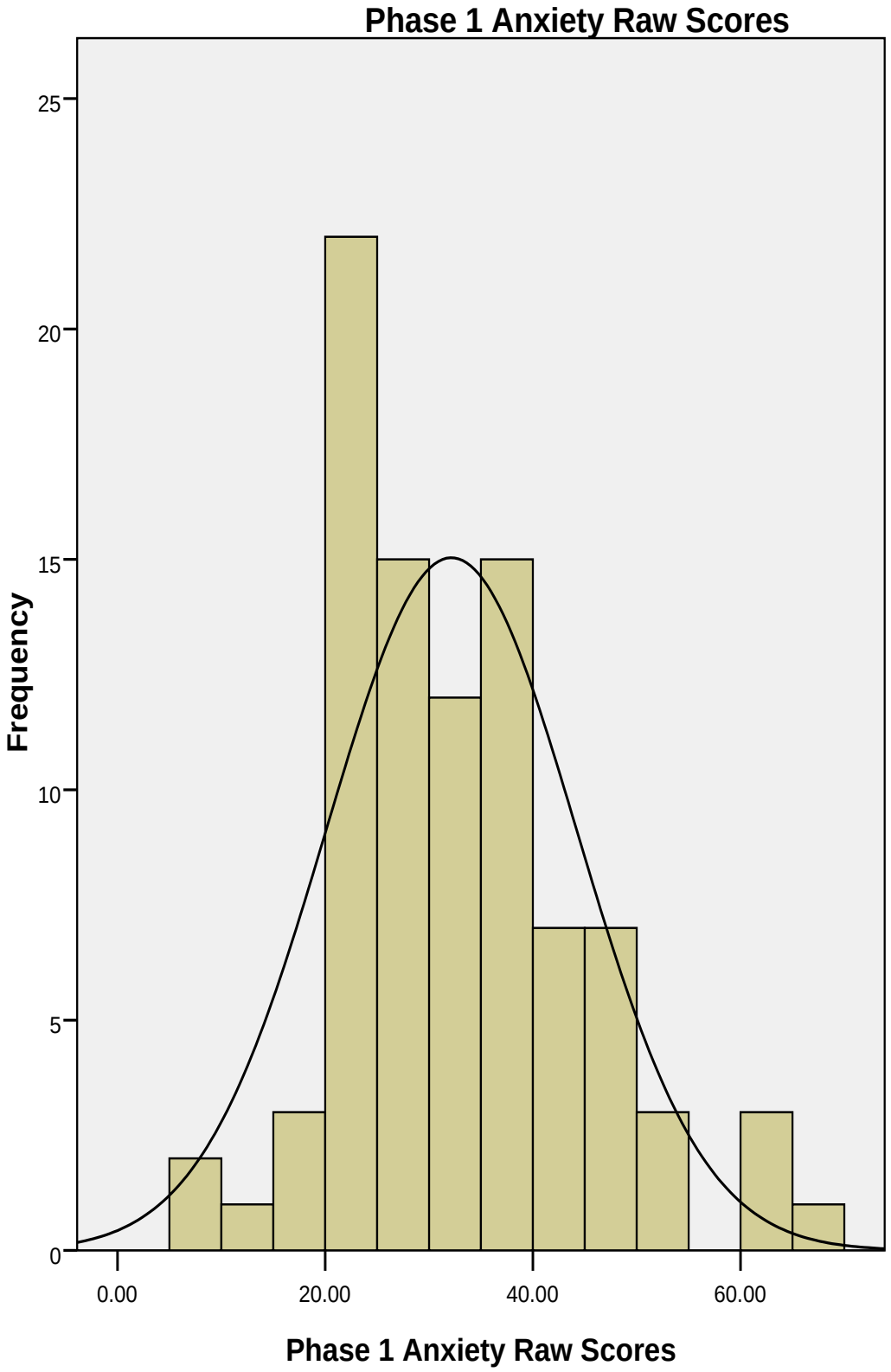
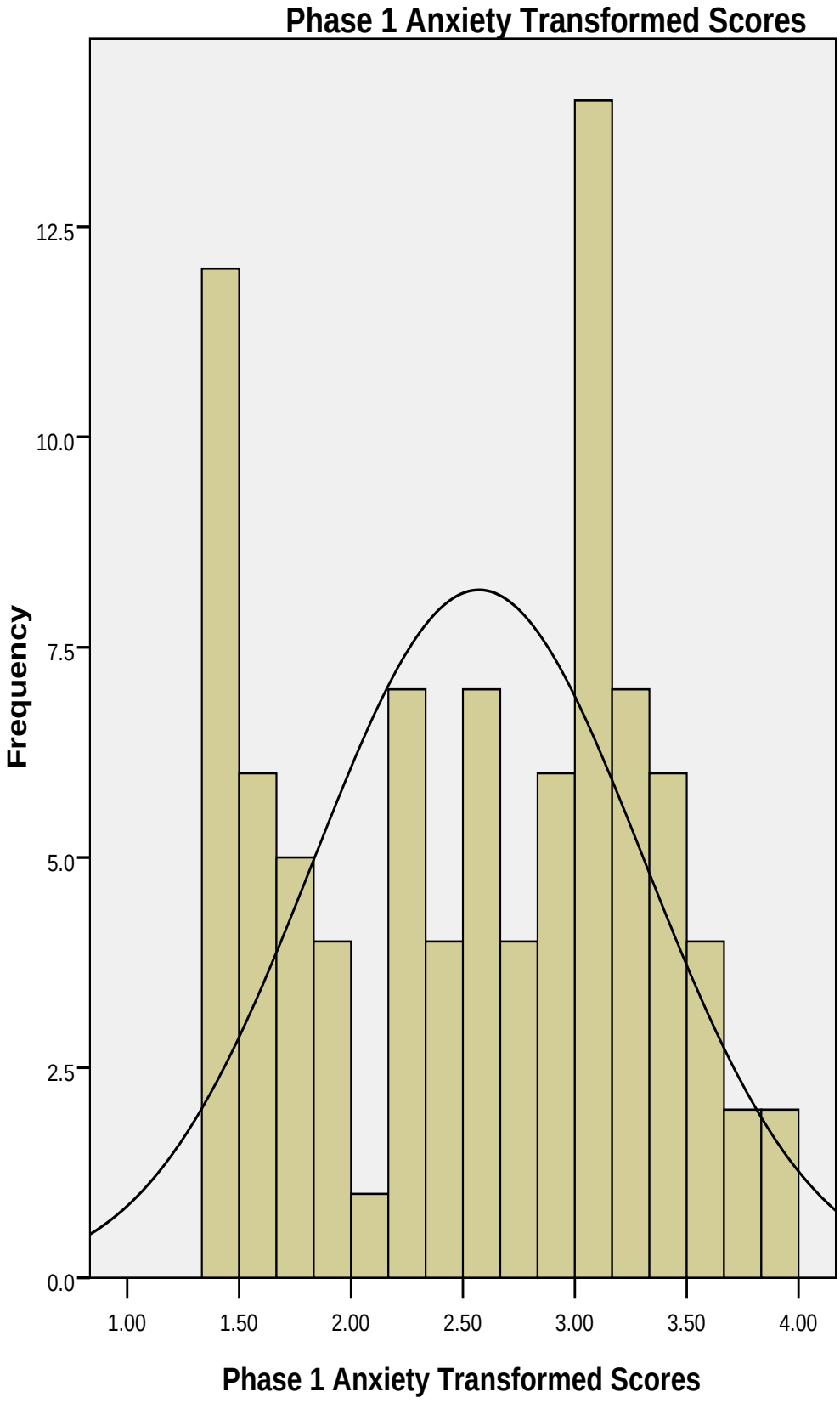
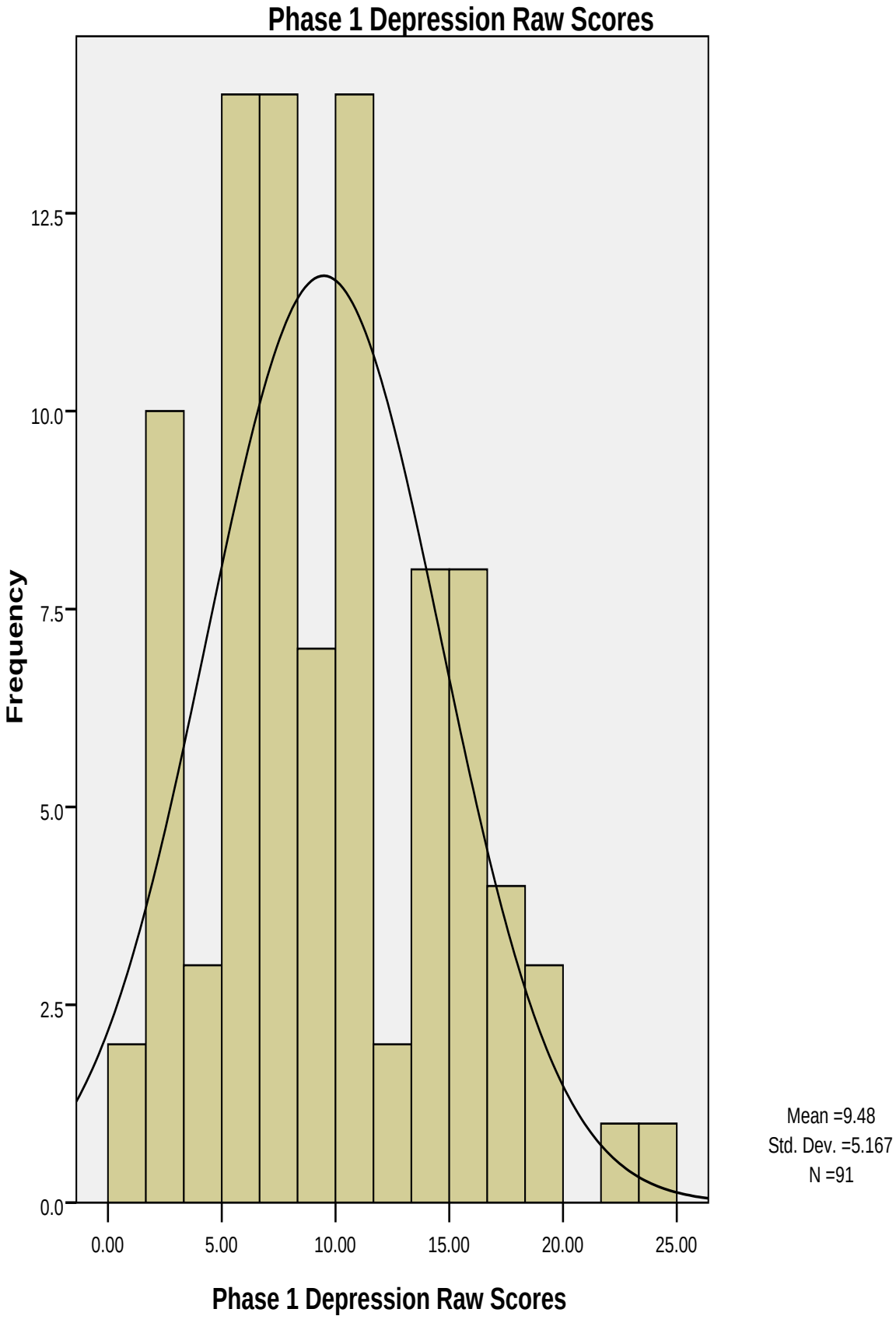
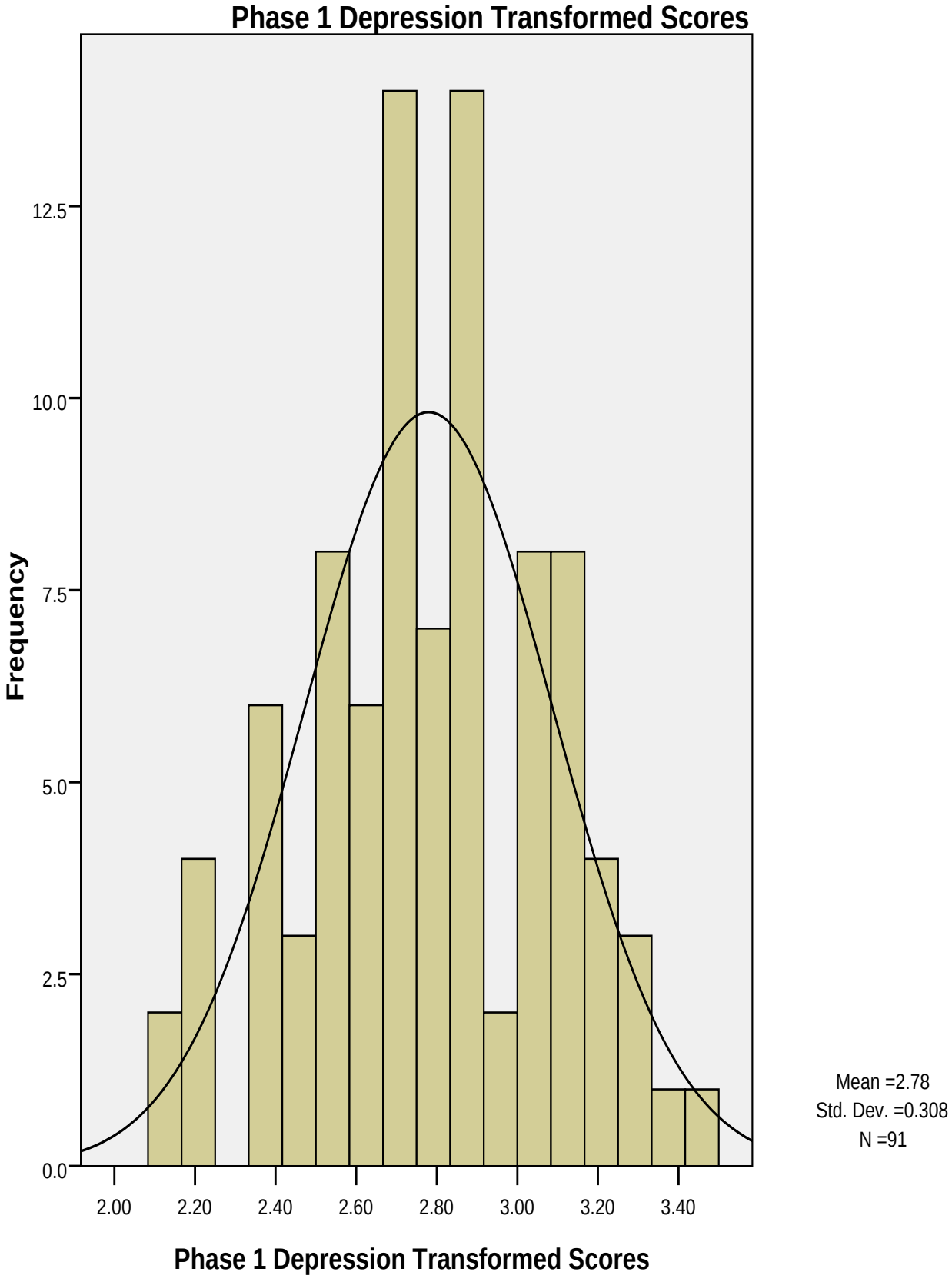
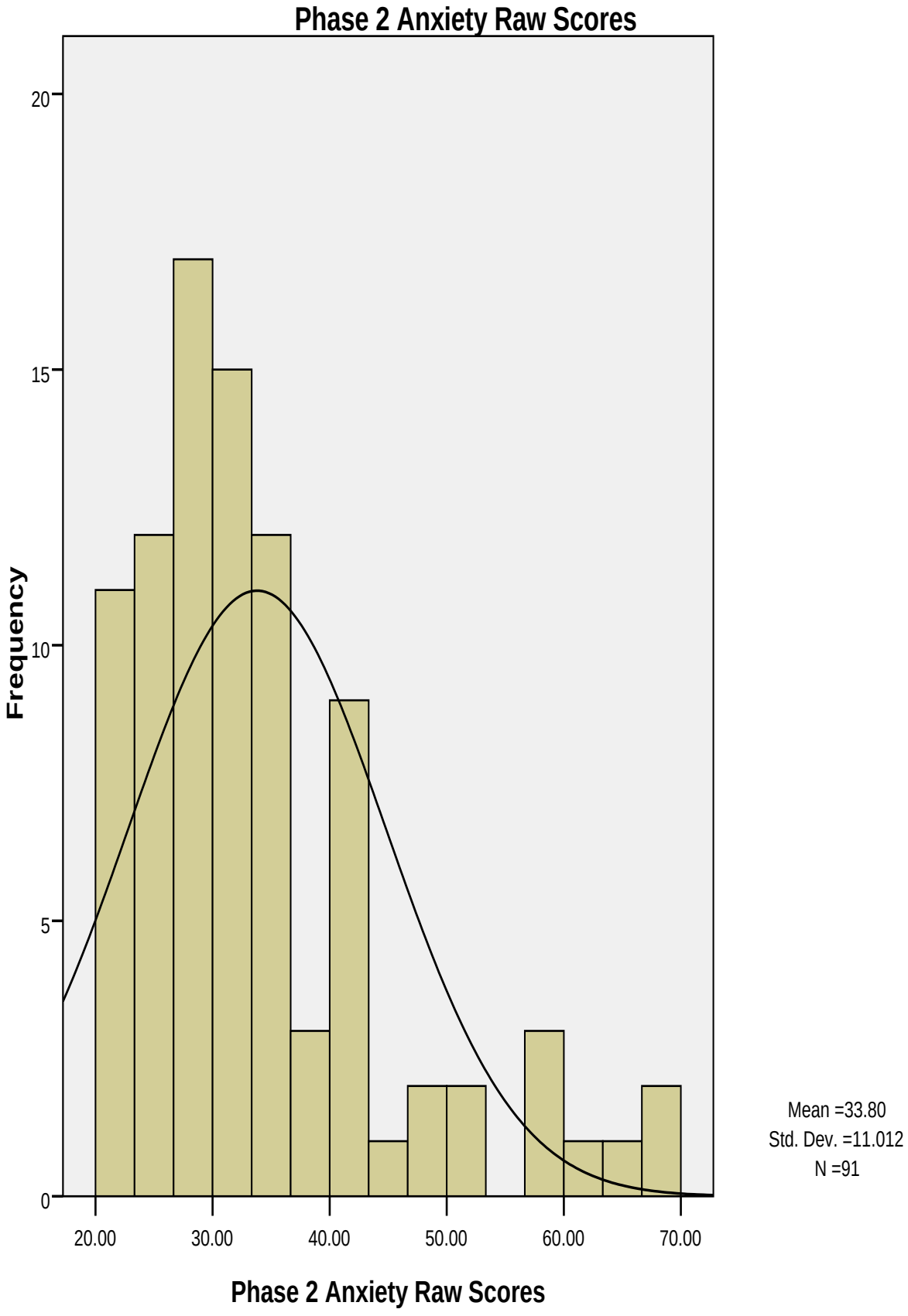
Appendix 1 Transformed and non transformed Phase 1, 2 and 6 maternal anxiety and depression scores

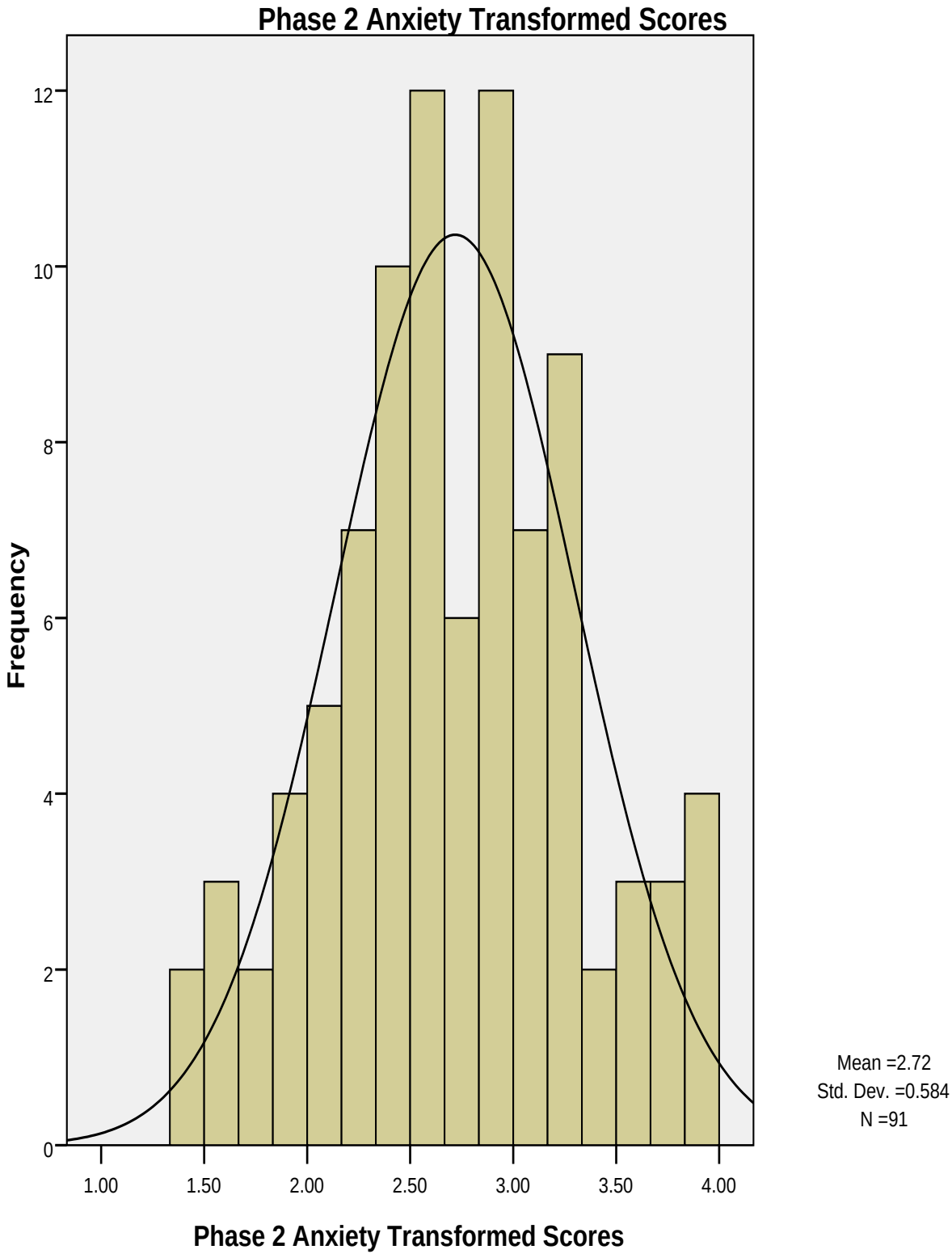


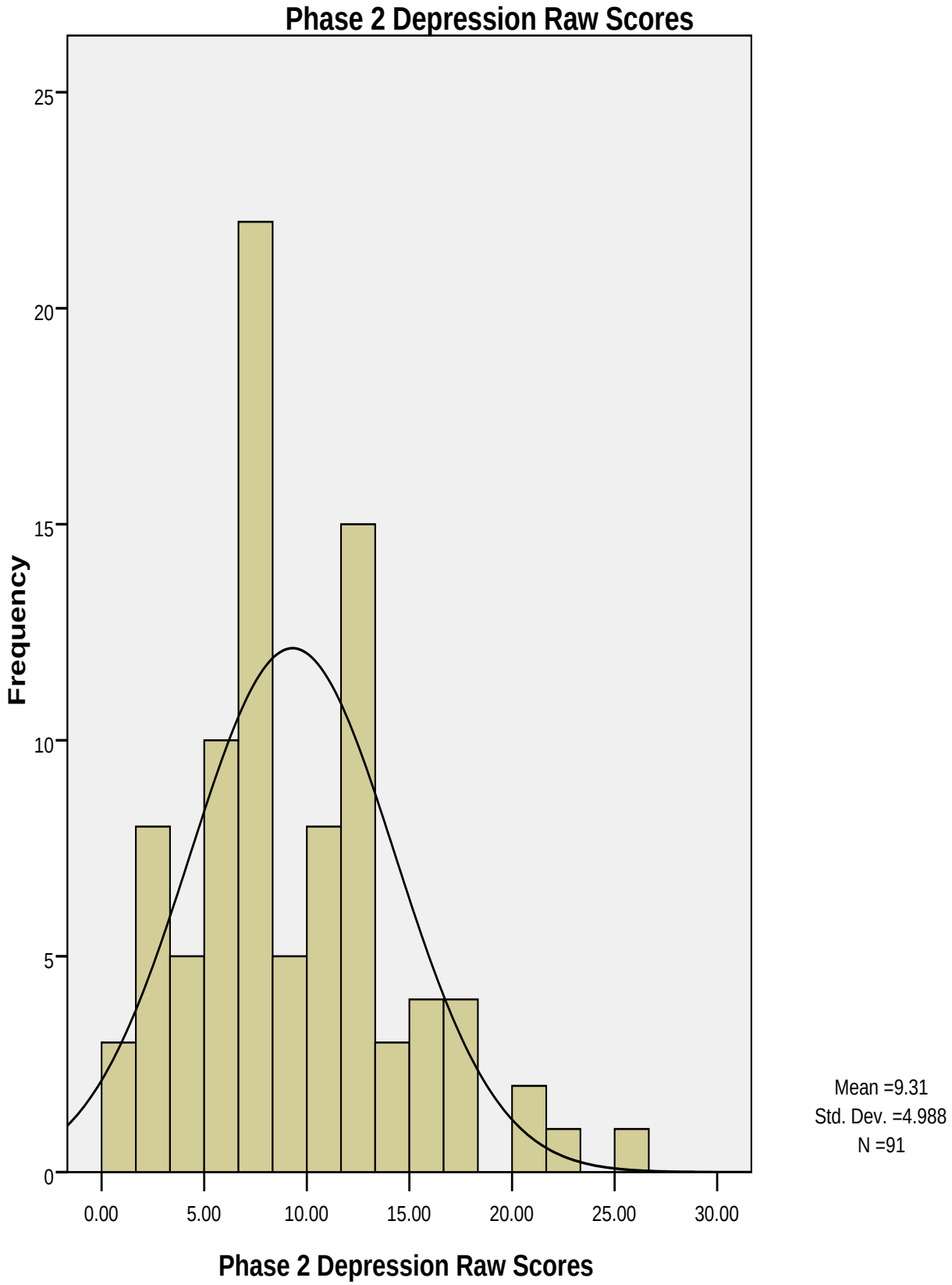

Phase 1 Anxiety Transformed Scores
Frequency
12.5
10.0
7.5
5.0
2.5
0.0
1.00
1.50
2.00
2.50
3.00
3.50
4.00
Phase 1 Anxiety Transformed Scores
Mean =2.57
Std. Dev. =0.74
N =91

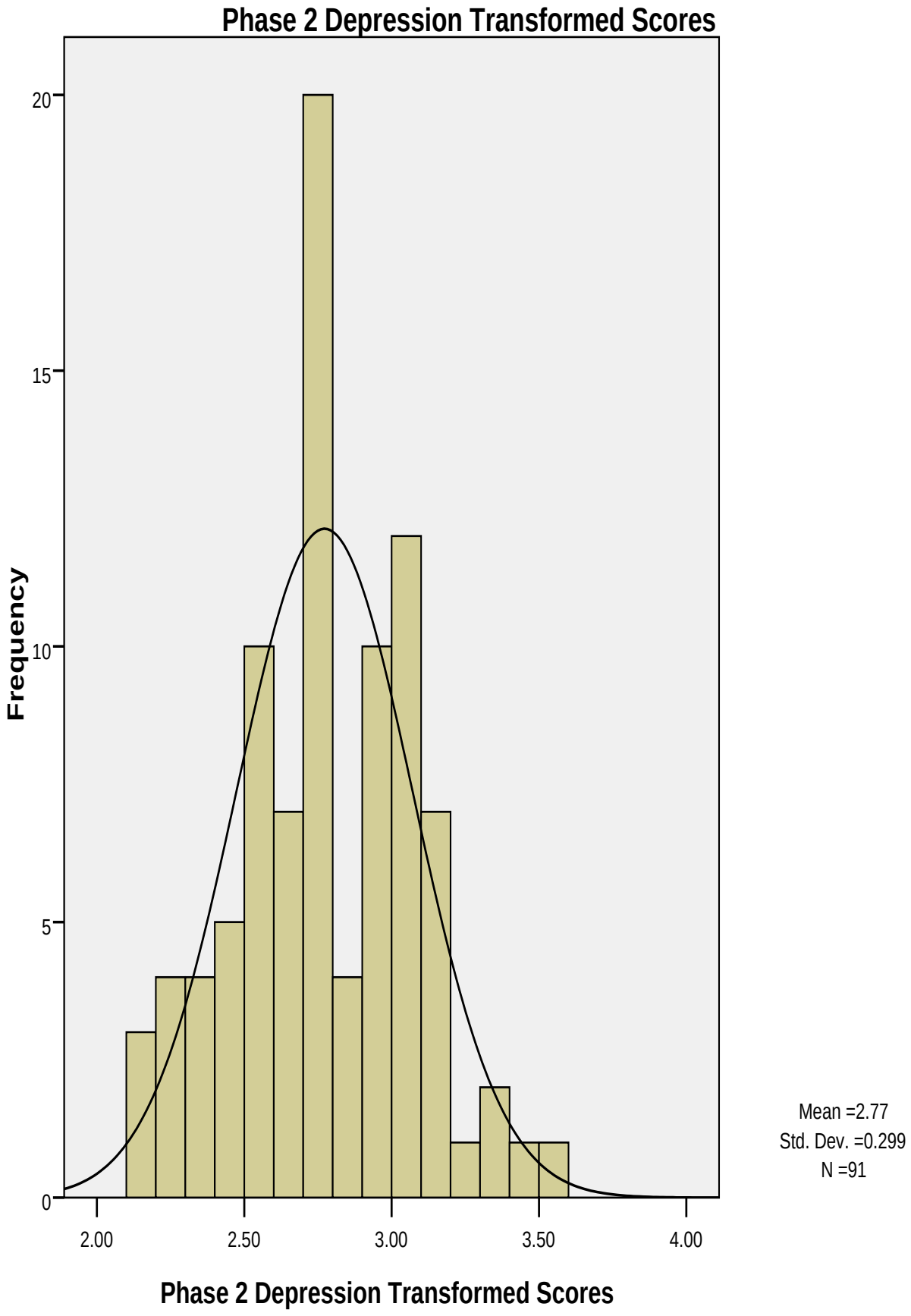

Phase 1 Depression Raw Scores
Frequency
12.5
10.0
7.5
5.0
2.5
0.0
0.00
5.00
10.00
15.00
20.00
25.00
Phase 1 Depression Raw Scores
Mean =9.48
Std. Dev. =5.167
N =91

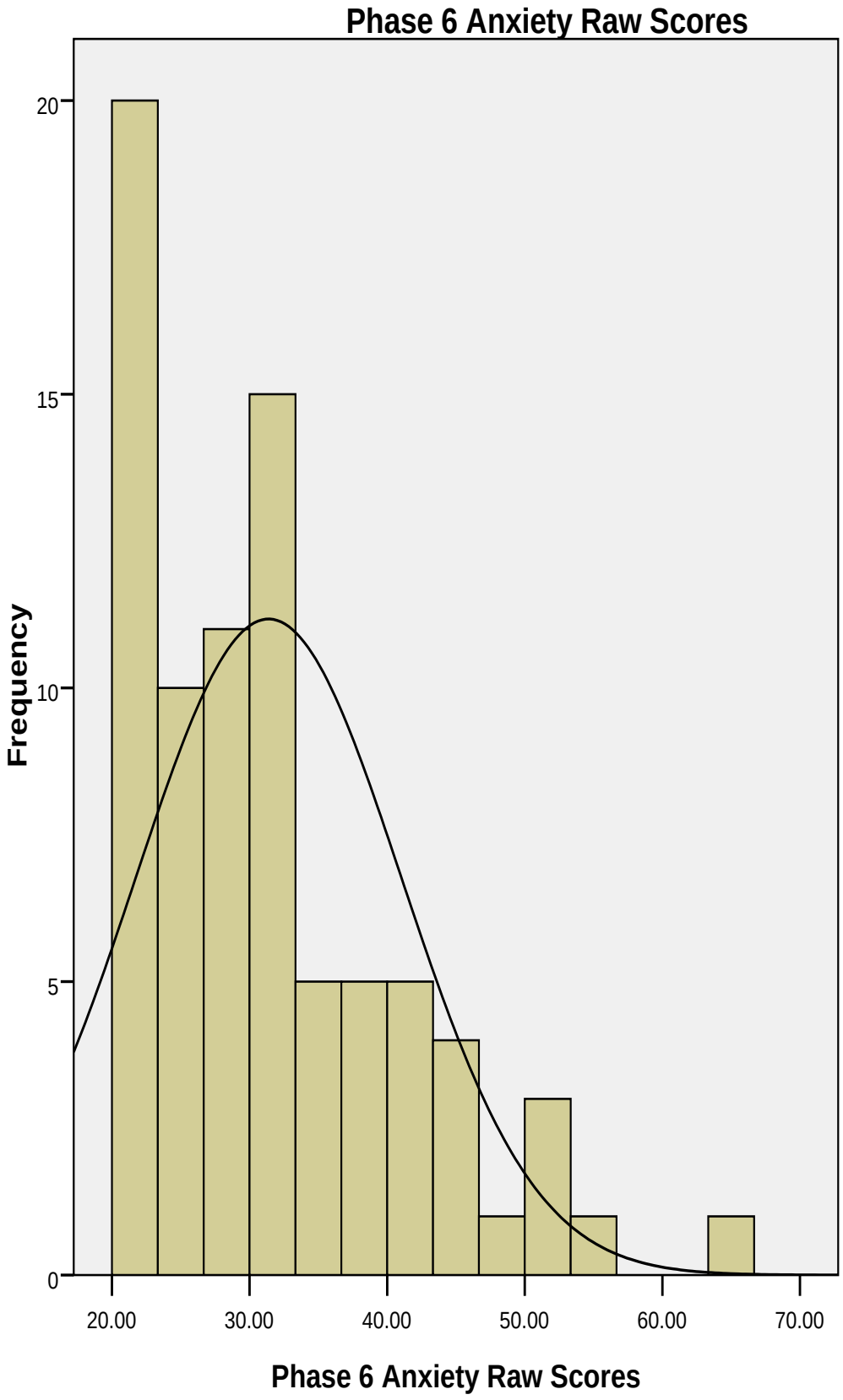

Phase 1 Depression Transformed Scores
Frequency
12.5
10.0
7.5
5.0
2.5
0.0
2.00
2.20
2.40
2.60
2.80
3.00
3.20
3.40
Phase 1 Depression Transformed Scores
Mean =2.78
Std. Dev. =0.308
N =91

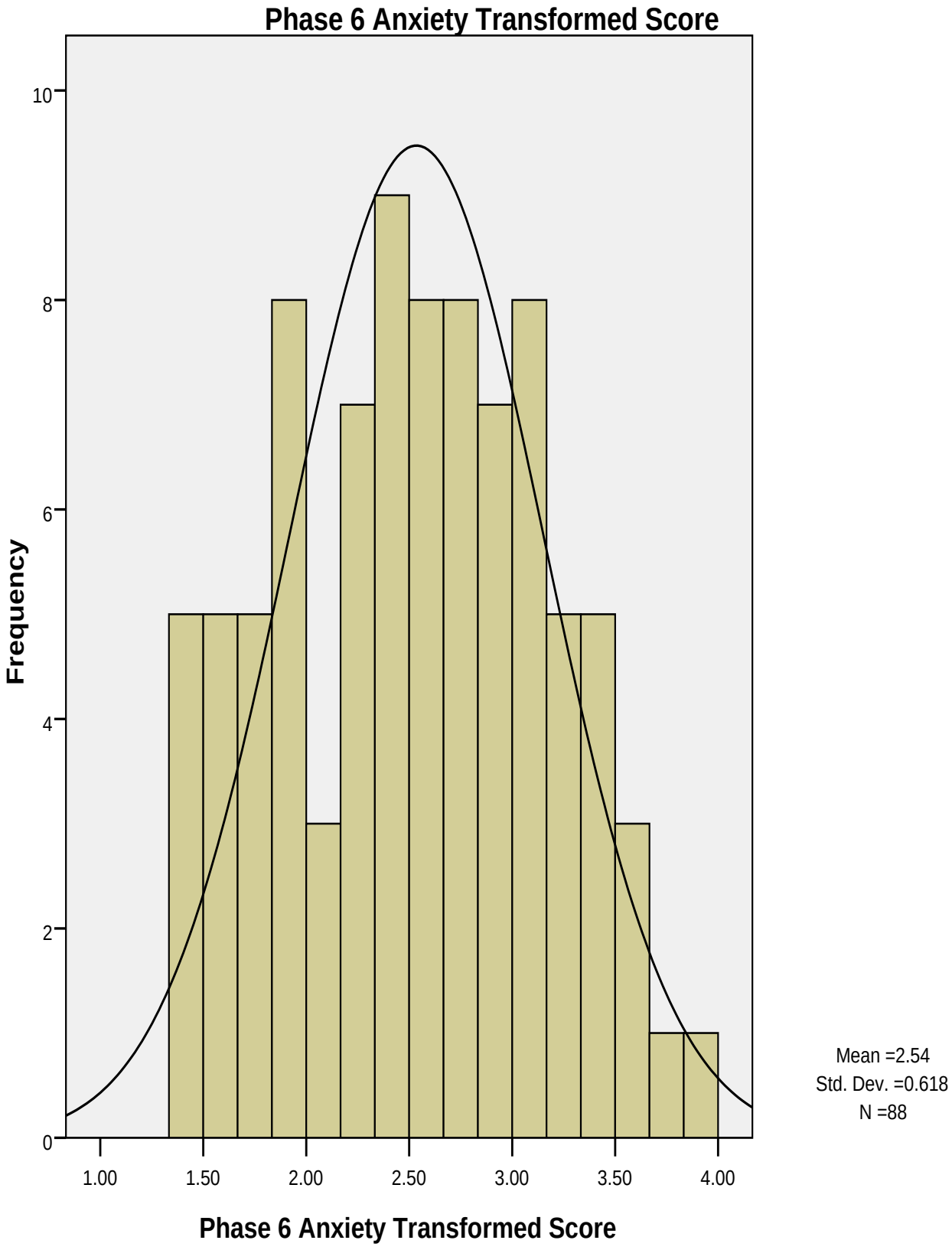

Phase 2 Anxiety Raw Scores
Frequency
20
15
10
5
0
20.00
30.00
40.00
50.00
60.00
70.00
Phase 2 Anxiety Raw Scores
Mean =33.80
Std. Dev. =11.012
N =91

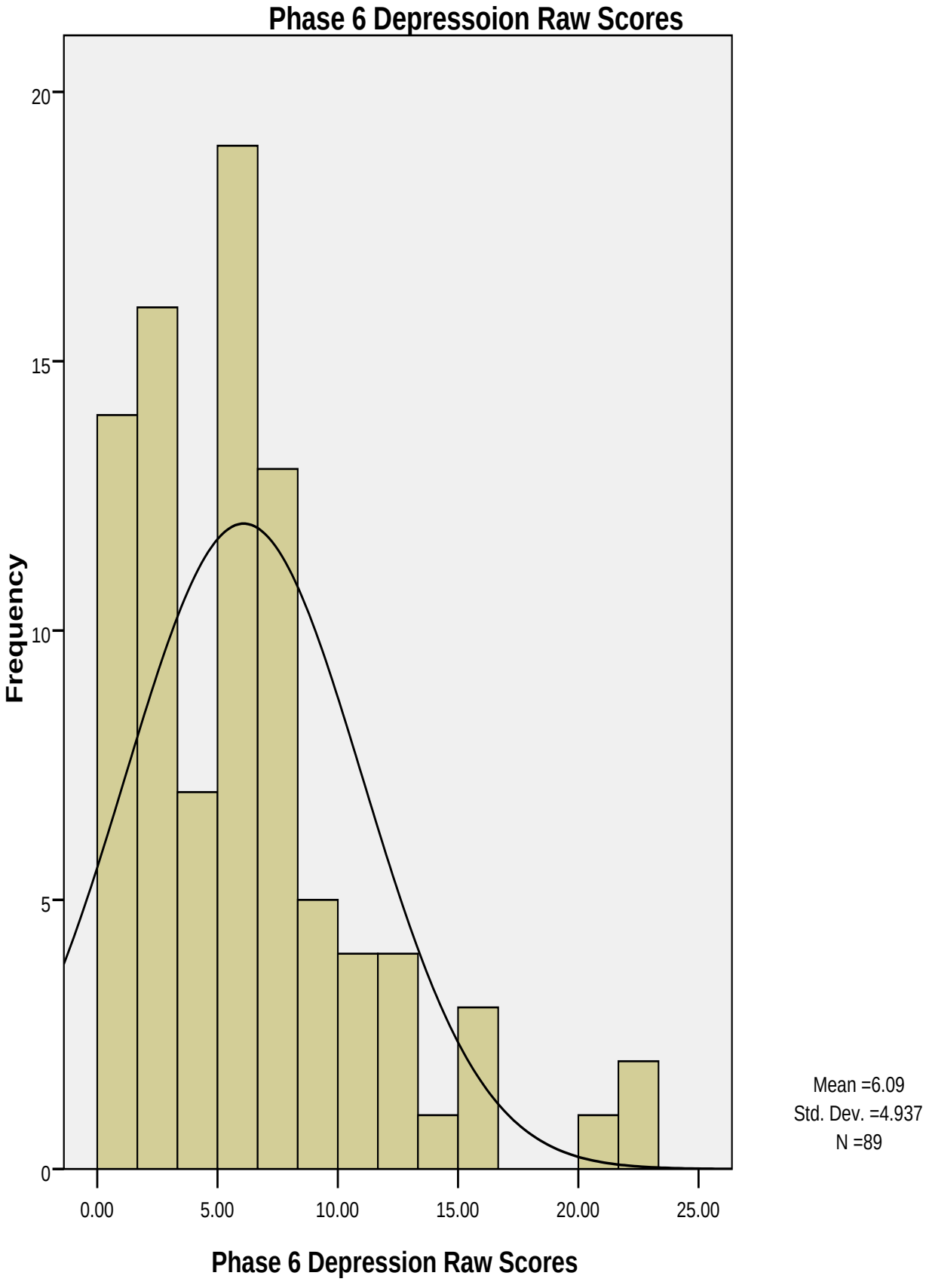

Phase 2 Anxiety Transformed Scores
Frequency
0
2
4
6
8
10
12
1.00
1.50
2.00
2.50
3.00
3.50
4.00
Phase 2 Anxiety Transformed Scores
Mean =2.72
Std. Dev. =0.584
N =91

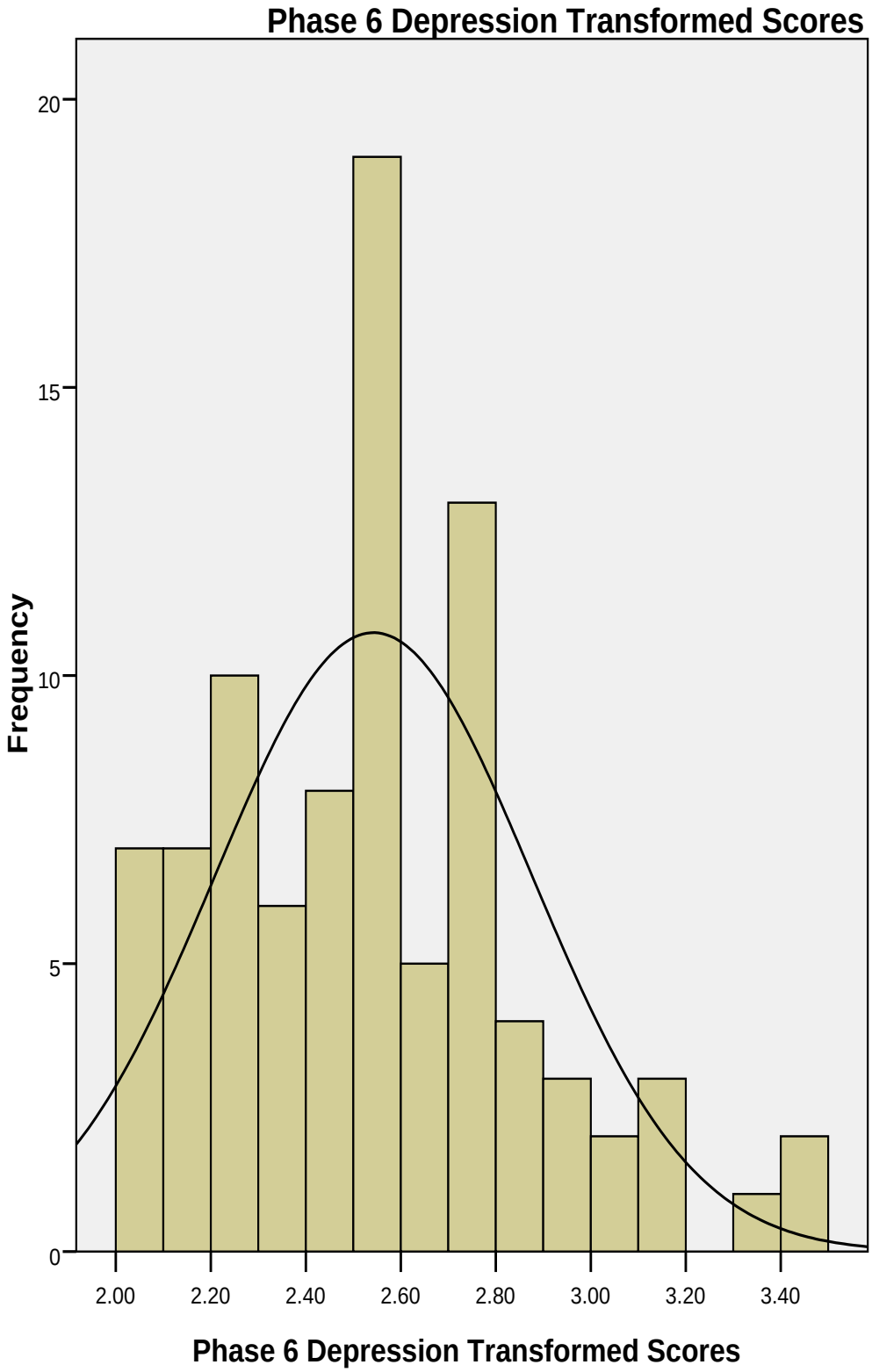

Phase 2 Depression Raw Scores
Frequency
25
20
15
10
5
0
0.00
5.00
10.00
15.00
20.00
25.00
30.00
Phase 2 Depression Raw Scores
Mean =9.31
Std. Dev. =4.988
N =91


Phase 2 Depression Transformed Scores
Frequency
20
15
10
5
0
2.00
2.50
3.00
3.50
4.00
Phase 2 Depression Transformed Scores
Mean =2.77
Std. Dev. =0.299
N =91

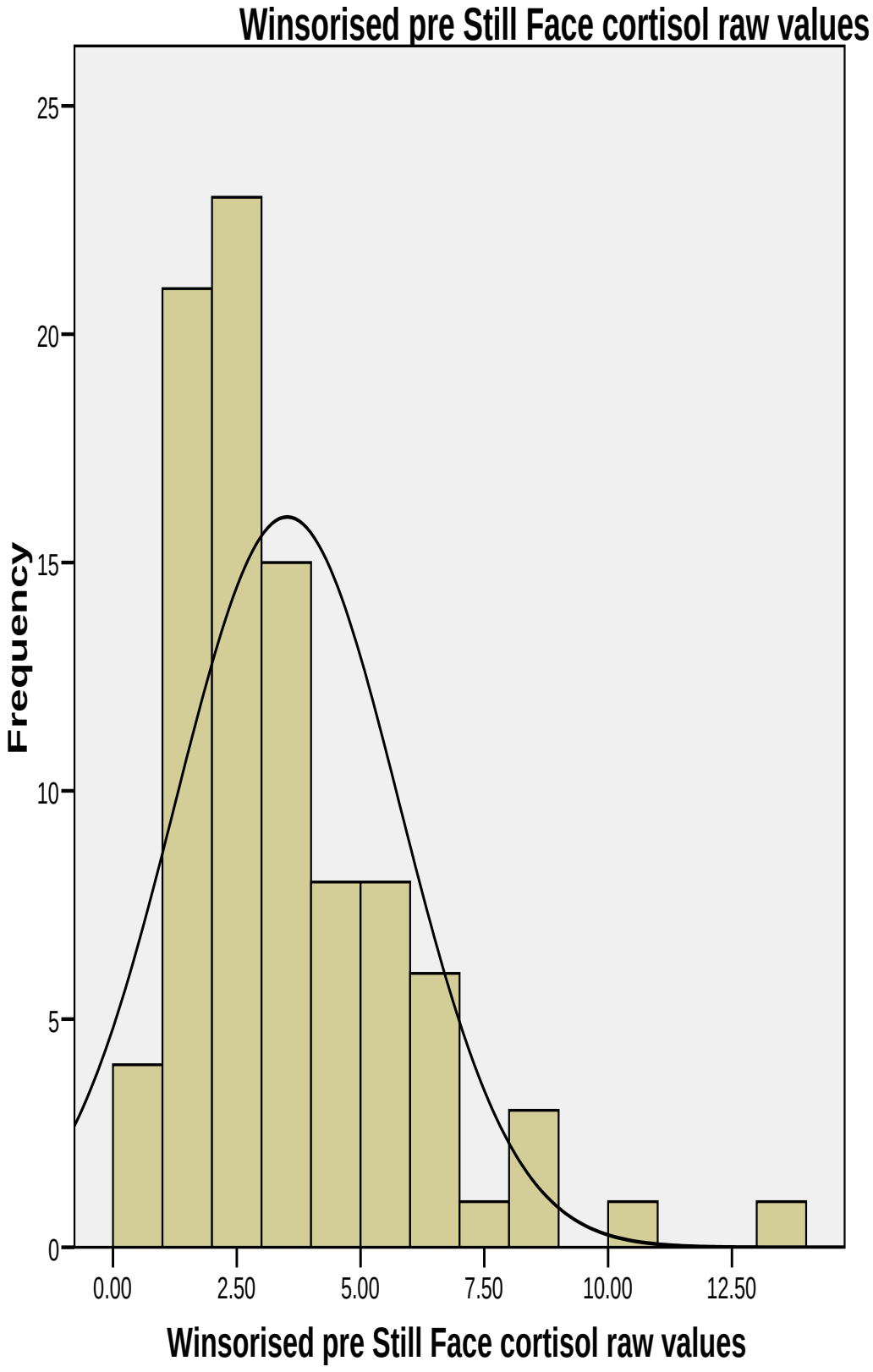

Phase 6 Anxiety Raw Scores
Frequency
20
15
10
5
0
20.00
30.00
40.00
50.00
60.00
70.00
Phase 6 Anxiety Raw Scores
Mean =31.38
Std. Dev. =9.64
N =81


Phase 6 Anxiety Transformed Score
Frequency
10
8
6
4
2
0
1.00
1.50
2.00
2.50
3.00
3.50
4.00
Phase 6 Anxiety Transformed Score
Mean =2.54
Std. Dev. =0.618
N =88


Phase 6 Depressoion Raw Scores
Frequency
20
15
10
5
0
0.00
5.00
10.00
15.00
20.00
25.00
Phase 6 Depression Raw Scores
Mean =6.09
Std. Dev. =4.937
N =89


Phase 6 Depression Transformed Scores
Frequency
20
15
10
5
0
2.00
2.20
2.40
2.60
2.80
3.00
3.20
3.40
Phase 6 Depression Transformed Scores
Mean =2.54
Std. Dev. =0.334
N =90

Appendix 2 Transformed and non transformed winsorised cortisol values



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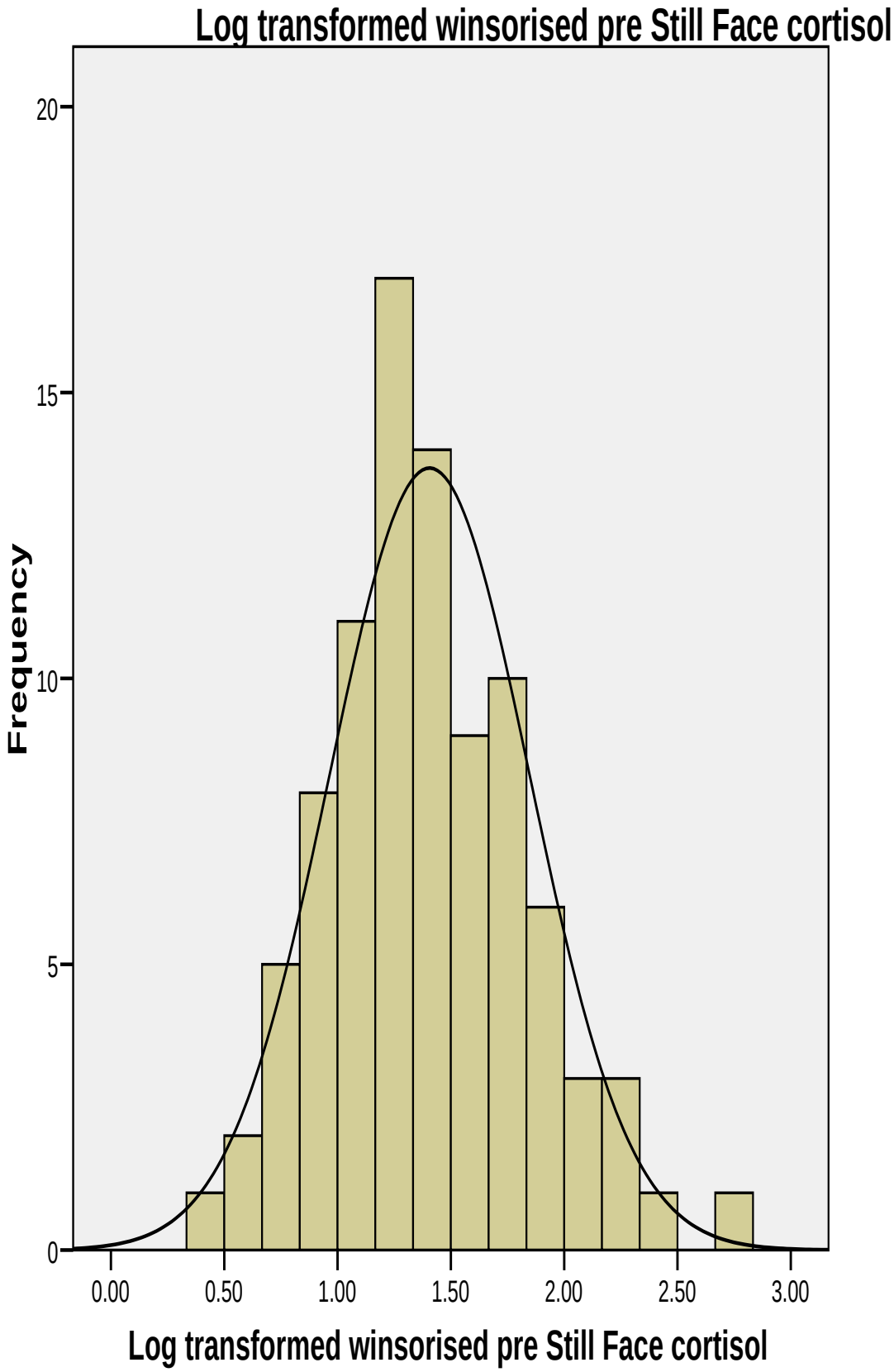
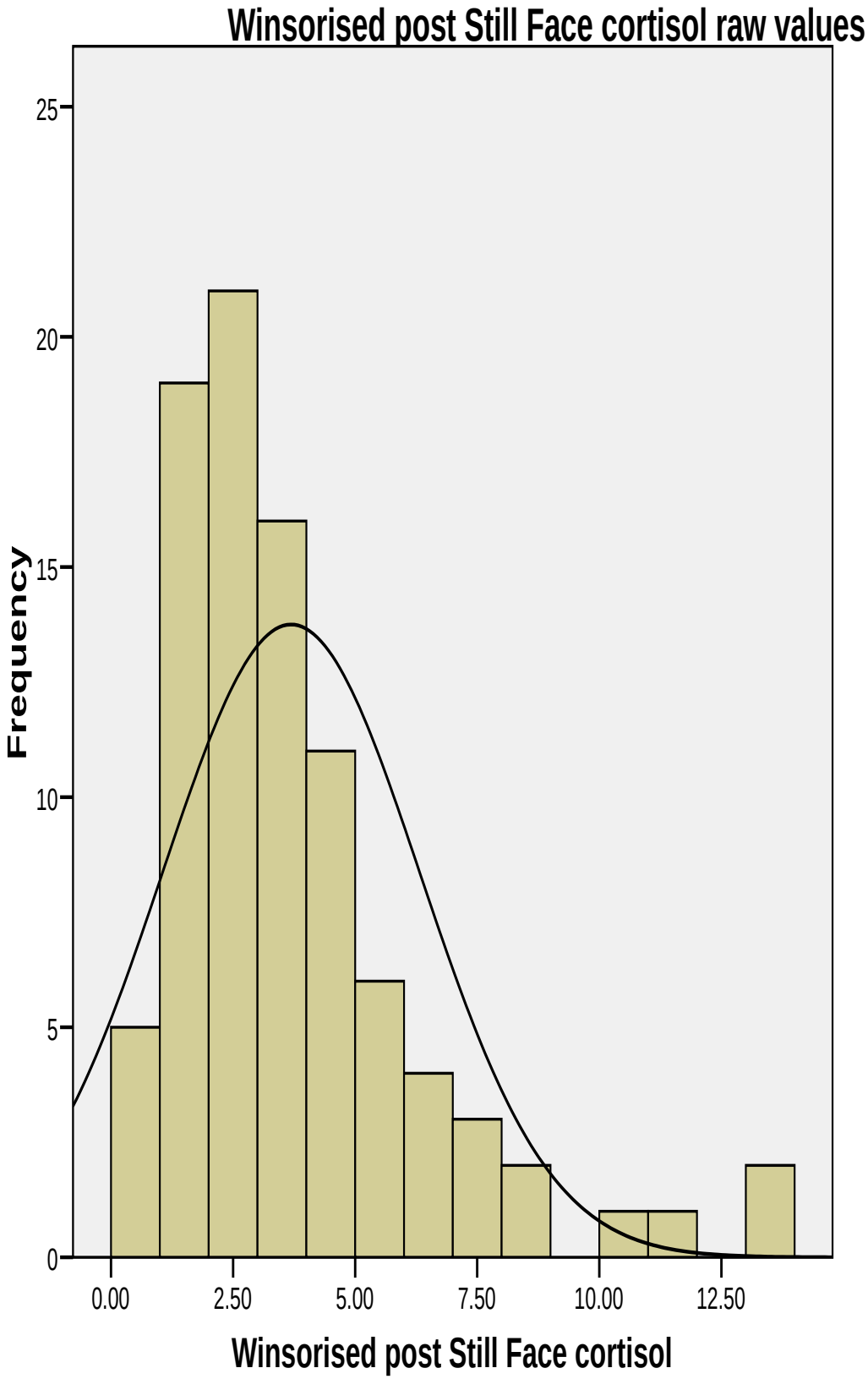
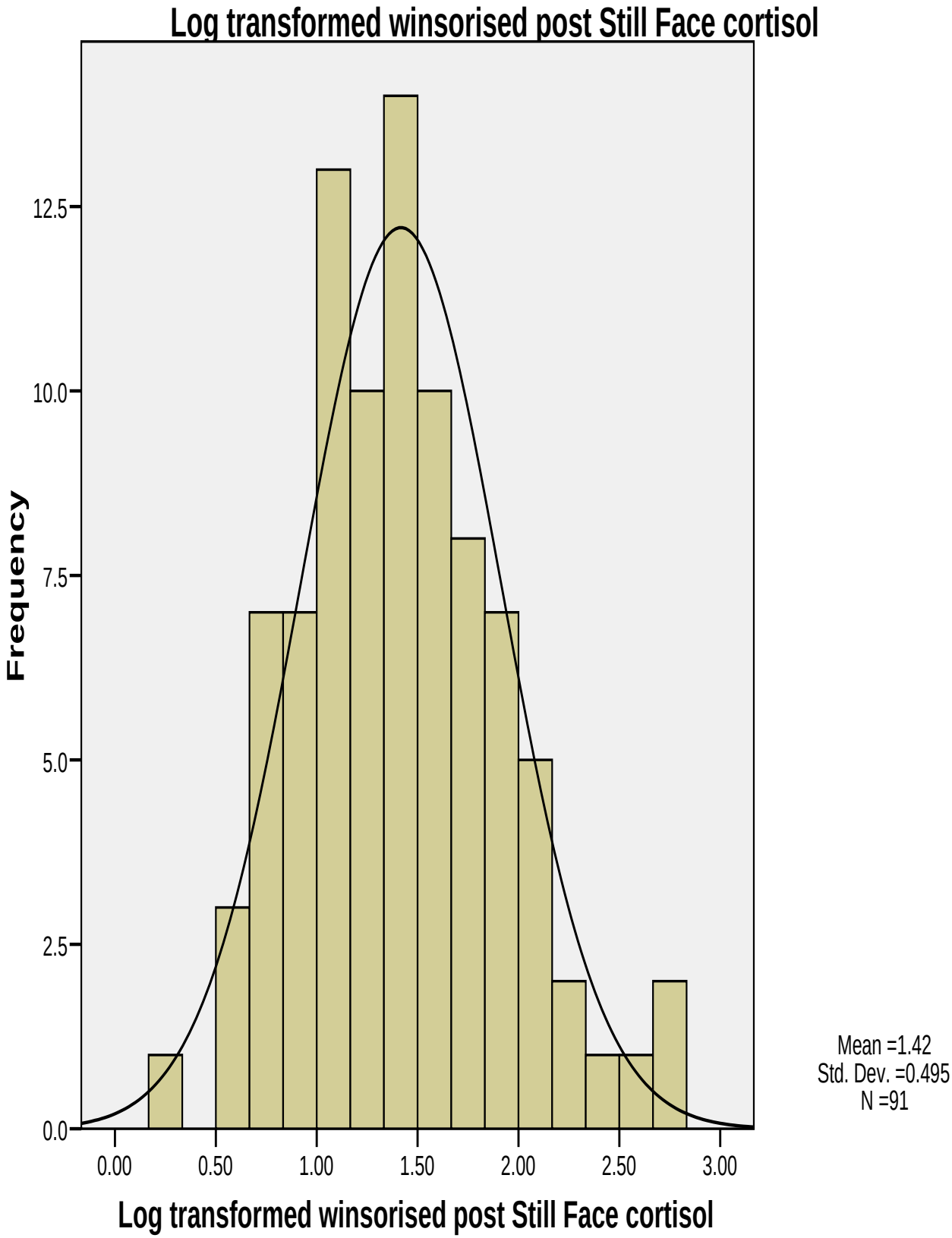

Log transformed winsorised pre Still Face cortisol
Frequency
20
15
10
5
0
0.00
0.50
1.00
1.50
2.00
2.50
3.00
Log transformed winsorised pre Still Face cortisol
Mean =1.41
Std. Dev. =0.442
N =91


Winsorised post Still Face cortisol raw values
Frequency
25
20
15
10
5
0
0.00
2.50
5.00
7.50
10.00
12.50
Winsorised post Still Face cortisol
Mean =3.69
Std. Dev. =2.64
N =91


Log transformed winsorised post Still Face cortisol
Frequency
12.5
10.0
7.5
5.0
2.5
0.0
0.00
0.50
1.00
1.50
2.00
2.50
3.00
Log transformed winsorised post Still Face cortisol
Mean =1.42
Std. Dev. =0.495
N =91

Appendix 3a

CREC Ethical approval letter (27th June 2006)

Appendices not available electronically

Appendix 3b

CREC Ethical approval letter amendment 1 (20th July 2007)

Appendices not available electronically

Appendix 3c

CREC Ethical approval letter amendment 2 (24th March 2009)

Appendices not available electronically

Appendix 4a

Mother information sheet and consent form, study 1500 - Phases 1, 3, 5 and 7

Appendices not available electronically

Appendix 4b

Parent information sheet and consent form, study 300 – Phases 2 and 4

Appendices not available electronically

Appendix 4c

Parent information sheet and consent form, study 300 – Phase 6

Appendices not available electronically