The Association between Maternal Distress and Serum Cortisol Levels in Children: Differential Outcomes by Asthma Diagnosis

by

Lisa C. Dreger

A Thesis Submitted to the Faculty of Graduate Studies

In Partial Fulfilment of the Requirements

For the Degree of

MASTER OF ARTS

Department of Psychology,

University of Manitoba

Winnipeg, Manitoba

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

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Abstract

Both asthma and exposure to maternal distress have been shown to correlate with altered cortisol levels in children, but my investigation of the relationship among all three variables appears to be a first. Using a representative sample (n = 503) of the 1995 Manitoba birth cohort, children's serum cortisol levels were examined in relationship to exposure to maternal distress and asthma status. A multiple linear regression analysis confirmed my hypothesis of a statistically significant interaction between childhood asthma and exposure to maternal distress in relation to children's serum cortisol levels. As predicted, non-asthmatic children exposed to recurrent maternal distress responded to an acute stressor with elevated cortisol levels whereas children with asthma responded with blunted cortisol levels. My results reinforce the need for mandatory postnatal screening of new mothers through programs such as Manitoba's Families First, which help at risk families in caring for their newborns and may prevent the dysregulation of children's stress response systems as well as conditions such as childhood asthma.

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Dedication

To Mike Hughes for his support and understanding throughout this endeavour

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As described in the Canadian asthma consensus guidelines (Becker, et al., 2005), asthma is a chronic disease that is characterized by periodic or persistent symptoms such as shortness of breath, tightness in the chest, wheezing, mucus production and coughing. These symptoms are associated with airway obstruction and airway inflammation (caused by an increase in airway responsiveness to a variety of stimuli). Presently, the main contributor to the development and persistence of asthma is considered to be changes in the structure of the respiratory tract due to inflammation of the airway tissue.

Asthma is becoming an ever growing health problem world-wide. In the Canadian population, the incidence of childhood asthma (under 14 years of age) has increased from a rate of 2.5% in 1978 to 11.2% in 1995 (Health Canada, 2001). A similar increase has been found in adults with rates of asthma having increased by 37% for women and by 33% for men aged 20-44 years between 1994 and 1999. In 1998/99, 7.5% of adults and 10.7% of children had an asthma diagnosis which amounted to a total of 2.5 million Canadians. Asthma has been reported as one of the top five reasons for hospital admission in Canada, accounting for 281,000 days in hospital and for 182,000 emergency room visits in 1990 (Krahn, Berka, Langlois, & Detsky, 1996). In 1990, the total cost to Canadian families due to asthma was estimated to be between 502 and 648 million dollars. Some of the costs calculated by Krahn et al. (1996) included such factors as: hospitalizations, emergency visits, physician services, drugs, disability payments, loss of productivity due to absence from work and school (for both patients and caregivers), and premature death. Drugs and inpatient care were reported to be the two largest contributors to the costs attributable to asthma.

All of the factors contributing to this complex, chronic illness are not yet completely understood. By gaining a better understanding of asthma and of the factors affecting its onset and persistence, we will become more capable of preventing new cases and of decreasing the severity of symptoms for affected individuals. Stopping, or preferably reversing, the increased incidence of asthma, will improve the quality of life for millions of people. It also would relieve some of the financial burden on health-care agencies worldwide.

Several lines of research are currently underway in an attempt to gain a better understanding of the mechanisms that underlie the symptoms of asthma. One approach, an epidemiological approach, has contributed greatly to our understanding of asthma. Through epidemiological research, a link between maternal distress and asthma symptoms in children has been discovered. For example, Sherwood-Brown, et al. (2006) reported 64% more asthma-related hospitalizations in children of mothers who had elevated scores on a self-report measure of somatic, anxiety, and depressive symptoms. A statistically significant, and potentially damaging, increase in cortisol levels found in children exposed to maternal distress also has been reported in the literature (e.g., Essex, Klein, Cho, & Kalin, 2002). Furthermore, a link between asthma and cortisol levels in children has been discovered which points to a relationship between asthma and the stress response system, since cortisol is considered to be a main physiological component of the human stress response (e.g., Buske-Kirschbaum, et al., 2003).

In my research, I examined the relationship between maternal distress and the functioning of the stress response system (measured by serum cortisol levels) in children with and without asthma. To set the stage, I will provide a brief account of the stress

response system and how serum cortisol levels can be used as an indicator of the functioning of this system. A review of the literature pertaining to the relationships between maternal distress and asthma in children, between maternal distress and children's cortisol levels, and between childhood asthma and cortisol levels will follow.

Cortisol is the glucocorticoid hormone produced by the hypothalamic-pituitary-adrenal (HPA) axis in humans in response to stressors. It is released according to a diurnal cycle in which concentrations peak 30-60 minutes after awakening in the morning (the awakening response) and decrease steadily throughout the day. The circadian release of cortisol can be disturbed by factors such as changes in lighting, eating schedules, and activity levels; but the most disruptive factor affecting this cycle is the presence of a stressor (Chrousos, 1997). During acute stress conditions, cortisol secretion is increased which serves the adaptive function of preparing the body for action (fight or flight response).

The following cascade of events occurs upon the introduction of an acute stressor (for a review of this system see Jacobson, 2005). The HPA axis is activated, causing the hypothalamus to produce corticotropin releasing hormone (CRH). The pituitary gland is, in turn, stimulated by CRH to produce adrenocorticotropic hormone (ACTH) which, when released into the bloodstream, stimulates the adrenal cortex to produce cortisol. A negative feedback mechanism exists within this system such that when cortisol is at a sufficiently high level in the bloodstream, the hypothalamus and the pituitary gland decrease the production of CRH and ACTH respectively. This, subsequently, reduces the production of cortisol.

Understanding how the neuroendocrine and neuroimmune systems are interconnected helps to explain the role that stress plays in chronic inflammatory diseases such as asthma. The production of cortisol by the neuroendocrine system upon the introduction of an acute stressor has an inhibitory effect on the immune system, particularly with respect to the production of cytokines, which are predominantly responsible for the inflammatory response. Thus, under stressful conditions (acute stress), the inflammatory response is typically suppressed. A bidirectional relationship exists among these two systems such that when cytokine levels are high (i.e., during an immune/inflammatory response), they trigger an increase in the release of cortisol by activating the production of ACTH which, as mentioned previously, mediates the production of glucocorticoids (cortisol). For a more detailed review of this relationship, please see Besodovsky and del Rey (2007) or Black (1994).

Under chronic stress conditions, when cortisol levels are elevated for a prolonged period of time, many harmful effects can occur, including an altered immune response (McEwan, B.S. 1998). After an extended period of stress, down-regulation of the HPA axis can occur, causing a decrease in the production of cortisol. This attenuation of cortisol results in an increase in the production of pro-inflammatory cytokines which are regulated by cortisol under normal conditions and this hypoactivity of the HPA axis has been implicated in the exacerbation of asthma symptoms under stressful conditions (Wright, Rodriguez, & Cohen, 1998). Another way that stressors can affect the immune system is through maladaptive coping behaviours such as smoking and drinking alcohol.

The measurement of cortisol levels is often used in research as a simple and convenient indicator of the functioning of the stress response system and is usually

conducted by analysis of saliva or blood serum samples. Salivary and serum cortisol levels in children have been found to correlate strongly (Burke et al., 1985) and, thus, the findings using both sampling methods can be combined in order to understand HPA axis functioning under various conditions. Single measurements of cortisol depend on many factors including time of day, the consumption of food or caffeine, tobacco usage, basal cortisol levels, the presence of infectious or inflammatory processes, the anticipation of a stressful situation, or taking a nap before the measurement is taken (Pollard, 1995). For these reasons, the collection of multiple measures has been recommended. Multiple samples allow for a comparison of the amount of variation within individuals to the amount of variation between individuals. Wust et al. (2000) suggest that the most reliable measure of HPA axis physiology is a daytime profile which involves collecting several measures for each participant throughout the day. However, they indicate that such measurement is expensive and time-consuming which makes it a difficult technique to use with large cohorts. They add that since cortisol is easy and inexpensive to collect and assess and since it is a consistent and reliable measure, it is useful, even if only a single measure is taken, for studying individual differences in HPA function in epidemiological studies involving large cohorts (Wust et al., 2000). No mention is made of the validity of a single measure.

With respect to inter-individual variation, several factors have been found to be associated with HPA axis activity. Age and gender are two major factors that must be taken into account when comparing cortisol levels among participants (Hruschka, Kohrt, & Worthman, 2005). Rosmalen et al. (2005) examined individual differences in HPA axis physiology and the diurnal cycle of cortisol in relation to potential confounds in a

large cohort (n = 1768) of boys and girls aged 10-12 years. Children taking corticosteroid medication, which may affect cortisol levels, were excluded from the study. As expected, it was discovered that gender was a relevant factor, with girls having a higher cortisol awakening response. No effect on cortisol levels was found in relation to birth weight, pregnancy duration, pubertal stage, or body composition. Age also was not found to be a relevant factor but the unexpected result is likely due to the small age range used. Wust et al. (2000), found that morning cortisol levels were not related to sleep duration, time of awakening, method of awakening (either spontaneously or by alarm), or differences in morning routines.

Considering this evidence in combination, it is reasonable to conclude that a single cortisol measure would provide sufficient representation of stress response functioning in a large cohort of children. Comparing the cortisol levels of children with and without asthma and then considering these data in conjunction with the presence of maternal distress should help to determine, if there is a relationship among the variables. A relationship among these three variables, considered together in a single study, does not appear to have been reported in the literature.

Literature Review

Maternal distress and cortisol levels in children

Maternal distress is a potent psychological stressor for children, as their stress response systems have been shown to be affected by exposure to it. Essex, Klein, Cho, and Kalin (2002), examined the effect of maternal distress during infancy, both alone and in combination with later maternal distress (during the preschool years), in relation to the

children's HPA axis activity during the preschool years. Maternal reports of stress were obtained at 1, 4, and 12 months and at 4.5 years in a sample consisting of 282 children. Maternal distress was determined by self report and consisted of symptoms related to depression, stress, and anxiety. The children's cortisol levels (adjusted for gender, time of day, and medications) were assessed at 4.5 years old using saliva samples that were taken for 2-3 days between 3 pm and 7 pm (before dinner) when the circadian cycle is at its least active point. The results indicated that children's exposure to maternal distress during the first year of life combined with maternal distress during the pre-school years was associated with reliably increased cortisol levels at pre-school age. The component of maternal distress that was most predictive of an increase in childhood cortisol levels was depression that began in the first year of life. Exposure to maternal depression during infancy (during the first year of life) in combination with exposure during the preschool years was most significantly associated with elevated cortisol levels at 4.5 years of age. The results indicated, then, that early exposure to maternal distress may sensitize children to subsequent stress exposure, since the children exposed to maternal stress during the preschool years had increased cortisol levels (and later mental health symptoms), only if they had a history of early stress exposure. These investigators utilized self-report measures which are susceptible to response biases and, thus, to measurement error. Using physician diagnoses and/or prescription records would provide more conservative results, but would be a superior measure to the self-reporting methods used. Halligan et al. (2004) used a physician diagnosis of postnatal depression rather than a self-report based measure and discovered that adolescent children of postnatally depressed mothers (diagnosed with postnatal depression when the children

were 6 weeks of age), displayed significantly higher baseline cortisol levels than control children of the same age with no history of exposure to maternal postnatal depression.

Essex et al. (2002) included socioeconomic status (SES) as a variable in their study of the effects of maternal distress on children's cortisol levels. A statistically significant main effect for SES on cortisol levels was found. Children living in low SES conditions had significantly higher cortisol levels than those living in higher SES conditions; however, those children living in lower SES conditions were exposed to higher levels of maternal distress both during the first year of life and at 4.5 years of age. The analysis suggested that the effect of exposure to maternal distress on children's cortisol levels is not due solely to its association with SES, since a statistically significant main effect was found for exposure to maternal distress that was independent of SES level. Similar findings were reported by Lupien, King, Meaney, and McEwen (2000) who also sought to establish whether there is a relationship between maternal distress, SES, and HPA functioning in children. A statistically significant relationship between SES and children's cortisol levels was found whereby children of low SES had higher cortisol levels than those of high SES. Lupien et al. discovered that low SES children were exposed to higher levels of maternal distress, and concluded that the independent effects of SES and maternal distress on children's cortisol levels could not be clearly identified. These findings, taken together, suggest that exposure to maternal distress during infancy, and particularly to maternal post-natal depression, may increase the vulnerability of the developing child's stress response system to later stress exposure, regardless of SES.

Relation between maternal distress and asthma

Wright et al. (2002) discovered an association between parental stress (96.6% maternal distress) and the onset of wheezing in children. Parental stress was assessed during seven bi-monthly interviews using a self-report measure of perceived stress starting when the infants were 2 – 3 months of age. Considering these results, it seems plausible that psychosocial stressors trigger the release of stress hormones (e.g., cortisol) in the early months of life which may influence the production of immune cells, perhaps via their effect on the production of cytokines which are thought to influence development of the immune system. Sandberg and Licinio (2000) found that chronic stress throughout childhood (family discord, poor parenting, inadequate living conditions, school bullying, and the chronic illness of a family member, including substance abuse) was associated with an increased risk of asthma exacerbations in children aged 6 – 13 years.

In contrast, Horwood, Fergusson, and Shannon (1985) found no correlation between childhood onset asthma and maternal depression; however, maternal depression was measured, not during infancy, but when the children were 5-6 years old. This suggests that postnatal maternal distress may be an important determinant in the development of asthma which is in keeping with the finding by Essex et al. (2002) that children's cortisol levels at pre-school age (4.5 years old) are primarily affected by exposure to maternal distress during infancy.

In a study of children genetically at-risk for developing asthma (n = 150), Klinnert et al. (2001) found that mothers' parenting difficulties in the first year of life was one of two variables that best predicted asthma at ages 6 to 8 years. Parenting difficulties were

defined as mothers' self-reported problems with infant care-giving, post-partum depression, and inadequate marital support. The second predictive variable was elevated levels of serum immunoglobulin E (IgE), an antibody associated with an allergic immune response. It also was discovered that a high number of respiratory infections during the first year of life significantly correlated with parenting difficulties in the first year of life which may help to explain previous reports indicating that a high number of respiratory infections early in life predicts childhood onset asthma (Mrazek et al., 1999). The correlation found between parenting difficulties and the number of respiratory infections in early childhood may be the result of the immunosuppressive effects on the stress response system in children of distressed mothers.

Klinnert et al. (2001) indicated that the course of asthma is affected by parenting difficulties in early life independently of possible related factors such as lower SES, exposure to maternal cigarette smoke, or duration of breastfeeding. It was reported that SES was not associated with asthma at ages 6 – 8 years, but lower SES was significantly correlated with the mothers' reported parenting difficulties, with a shorter duration of breastfeeding, and with maternal smoking. It was suggested that the lack of a correlation between SES and asthma at ages 6 - 8 years was due to the small number of cohort children from lower SES families in the study and should not be considered to generalize to the greater population. At 6 – 8 years of age, the cohort children's internalizing scores on the Child Behavior Checklist and overall scores on the Child Psychological Risk test were reliably correlated with concurrent scores of maternal depression. Since cortisol levels are known to correlate with psychological dysfunction, this finding supports the

idea that the children's psychological functioning and presumably their stress response systems were affected by the psychological stressor of having a distressed mother.

Using a large sample of children (n = 496) who were genetically predisposed to asthma, Wright et al. (2002) found that higher levels of self-reported distress in mothers was associated with the risk of recurrent early childhood wheeze. Higher levels of perceived stress at the time of the first measure (at 2-3 months of age) corresponded to an increased risk of repeated wheeze in the children during the first 14 months of life. This relationship was found to be independent of possible confounding variables such as birth weight, parental asthma, and SES. An increased risk of wheeze in the children was predicted by high levels of maternal stress, even after controlling for the effects of maternal stress-related behaviours such as exposure to environmental tobacco smoke (maternal smoking), home allergen levels, and length of breast feeding which suggests that the relation between maternal distress and early childhood wheeze may not be mediated directly through these behaviours. The relationship also remained when the number of lower respiratory tract infections was controlled. In a related study, Wright et al. (2004) found that chronic maternal stress was predictive of higher levels of IgE expression and altered cytokine expression in children which is consistent with the theory that maternal distress contributes to the development of asthma through its effect on immune function.

Asthma and cortisol levels in children

A relationship between the presence of asthma in children and the functioning of their stress response systems is well documented. The relationship has been found to be one of hypoactivation of the HPA axis. Landstra, Postma, Boezen, and Van Aalderen

(2002) found that children with asthma had lower cortisol levels throughout the entire diurnal cycle than normal controls and Buske-Kirschbaum et al. (2003) discovered an attenuated cortisol response to an acute stressor in asthmatic children. Buske-Kirschbaum et al. (2003) compared age- and gender-matched children with and without asthma (n = 34) and found a significantly blunted cortisol response to an acute stressor (public speaking task followed by a math task) in the asthmatic children. From these as well as previous findings, the authors suggest that a blunted HPA axis response may be responsible for the abnormal immune/inflammatory reaction inherent to many children with asthma, particularly when the children are faced with stressful conditions. Hoglund et al. (2006) found similar results in a study on the effects of examination anxiety on the stress response (using urine cortisol levels) of atopic vs. healthy university-student participants. Compared to the healthy controls, the atopic individuals responded with significantly lower cortisol levels during periods of stress.

It must be noted that the effects of corticosteroid medications (glucocorticoids) on HPA functioning has been vigorously studied and the results seem to indicate that the commonly prescribed asthma medications impair HPA axis activity when administered at moderate to high doses and after a prolonged period of use (e.g., Ninan et al., 1993; Brown, Blundell, Greening, & Crompton , 1991; Smith & Hodson, 1983; Dorsey et al, 2006). However, some research points to no effect (Brown et al., 1992) or to a mild effect (Kannisto, Korppi, Remes, & Voutilainen, 2000; Priftis et al, 2006) when medications are kept at clinically recommended doses, while others have discovered a suppression of HPA axis activity even at lower doses (Ozbek, Turkatas, Bakirtas, & Bideci, 2006; Masharani et al., 2005). As a precaution, participants were not included in the above

mentioned studies, if they had received corticosteroid treatment within 3 months of the studies. We are assured, then, that the blunted cortisol response observed in asthmatic children in these studies is not due to the use of glucocorticoid medication.

An explanation for this relationship between asthma and HPA functioning was offered by Chrousos (1997) who described the developmental periods of infancy, childhood and adolescence as times of increased brain plasticity (critical periods) in which abnormal activation of the stress response system can trigger a lifelong effect on its function and may potentially create a predisposition to stress- and immune-related conditions such as asthma. Consistent with this analysis, Wright, Rodriguez, and Cohen (1998) reported that children with asthma may have developed an atopic phenotype, as the result of a problem in the development of their immune system earlier in childhood. This possibility has stimulated research investigating the influence of early life environmental factors on the maturation of the immune system and on the development of asthma. An important early environmental factor that has been implicated is psychosocial stress.

One factor that is reported as evidence for the relationship between psychosocial stress and the immune system is the occurrence of respiratory tract infections in early childhood. Psychological stress is understood to be associated with the contraction of respiratory infections in childhood, and respiratory tract infections in early childhood are understood to be associated with the risk of developing asthma (Anderson, Bland, & Peckham, 1987). These associations offer a possible mechanism for the effect that psychological stress has on the development of asthma in children and this relationship may be mediated by maternal distress through the destructive factors that often

accompany a mother's distressed psychological state. Some such factors are the inadequate development of bonds with their children (attachment theory), the lower incidence of breast feeding, the higher likelihood of smoking, the reduced inclination to seek medical attention, and on a more practical note, the probability that they will be less concerned with eliminating potential allergy and asthma triggers by keeping up with regular cleaning. All of these factors affect children's well being, including the development of their stress response system and, as a result of them, children may be more susceptible to developing asthma.

In summary, previous research indicates that there is an association between maternal distress and children's stress response systems, and it specifically suggests that exposure to maternal distress increases cortisol levels in children (both at baseline, but also in response to an acute stressor). Another association that has been reported in the literature is the relationship between the exposure to maternal distress and the development of and exacerbation of childhood asthma symptoms. A third association that has been reported is one between cortisol levels and asthma. The research indicates that children with asthma have, not only lower baseline cortisol levels, but also that they demonstrate a blunted response to acute stressors. These relationships are well documented and seem to point to a difference in the effect of maternal distress on the stress response system for children with and without asthma. The possible interaction between childhood asthma and previous exposure to maternal distress, as they relate to children's cortisol levels at age 7 – 10 years was investigated specifically in my research.

Possible confounding variables

Apart from maternal distress and the presence of asthma, there are several potential confounding factors related to these variables that were considered in the analysis of children's stress response systems. As previously mentioned, maternal distress (depression and anxiety) is associated with an increased risk of smoking (Groer and Morgan, 2007; Lasser et al., 2000). Exposure to environmental tobacco smoke (ETS) has been linked to an increase in the incidence of asthma in children (Weitzman, Gortmaker, Walker, & Sobol., 1990; Polanska et al., 2006). The relationship between ETS and asthma may be due to the fact that exposure to ETS increases the rate of upper respiratory infections which, as mentioned earlier, are associated with the development of asthma. Furthermore, smoking is known to increase cortisol levels (Badrick, Kirschbaum, & Kumari, 2007; Steptoe & Ussher, 2006) and, since exposure to ETS has physiological effects similar to smoking first hand, it is possible that exposure to ETS results in a similar elevation of children's cortisol levels. Therefore, exposure to ETS during the first year of life was included in my research as a possible confounding variable.

Another potential confounding variable included in my study was low birth-weight, which has been associated with an increase in basal cortisol levels (Levitt et al, 2000) as well as with an elevated cortisol response to an acute psychosocial stressor (Wust et al., 2005). These effects reportedly remained statistically significant after controlling for both length of gestation and smoking. In addition to these reported effects on cortisol levels, a low birth weight also may be associated with asthma status in the children. As indicated by Hack et al. (2005), infants born with a low birth-weight have

an increased risk for developing asthma which may be due to the effect that an altered HPA axis has on the developing immune system.

Exclusive breast feeding is less likely to occur in postnatally distressed mothers (Pippins et al., 2006; McCarter-Spaulding & Horowitz, 2007) which may affect the development of the infant's immune system either directly through the lack of exposure to the immune enhancing properties of breast-milk or indirectly through the infant's stress response system which may be affected by the psychosocial stressor of an improper mother-infant attachment (Tarullo & Gunnar, 2006). For the reasons that failure to breast feed may be associated with cortisol levels, with maternal distress, and potentially with the development of asthma, the failure to exclusively breast feed for the child's first 12 weeks of life was included as a potential confounding variable.

Children may be born with a genetic predisposition to asthma (Morgan & Martinez, 1992; Gold & Wright, 2005; Mrazek et al., 1999). Children also may be genetically predisposed to irregular cortisol levels (Mannie, Harmer, & Cowen, 2007; Wust, Federanko, Helhammer, & Kirschbaum, 2000). Therefore, it is possible that the correlation that has been discovered between asthma and lower cortisol levels represents a common genetic predisposition. For this reason, a genetic predisposition to asthma, represented by a family history of asthma, was considered as a potential confounding variable due to its known association with childhood asthma and its possible correlation with abnormal cortisol levels.

The use of inhaled corticosteroids is common for children with an asthma diagnosis and may negatively affect children's cortisol levels. For example, Eid et al. (2002) found that 35% of children on a standard dose and 45% of children on a high dose

of inhaled corticosteroids exhibited abnormally low cortisol measures. Because the use of inhaled glucocorticoids is associated with an asthma diagnosis and may be associated with cortisol levels, it also was included as a possible confounding variable.

Purpose

As reported in the literature, maternal distress in early childhood is associated with an increased stress response (higher cortisol levels in response to an acute stressor) in later childhood and a correlation between maternal distress and the development of asthma in children also has been discovered. A relationship between asthma and the functioning of the stress response system (as measured by cortisol levels) has been reported as well, and the research indicates that children with asthma respond to acute stressors with blunted cortisol levels. In my research, I have investigated these relationships with a particular focus on the association between maternal distress and the stress response in children (as measured by cortisol levels) with and without asthma. I have attempted to determine whether asthmatic and non-asthmatic children of distressed mothers demonstrate differences in their stress response functioning.

Four main improvements over previous research have been instituted. First, it appears as though no prior study has included the three variables of maternal distress, children's asthma, and children's cortisol levels together within the same study. The second improvement upon previous research was the use of a more objective and presumably more reliable and valid measure of maternal distress. Rather than relying on self-report questionnaires, which are susceptible to response and recall biases, I have utilized physician diagnoses, as documented in the participants' medical records, as well as prescription histories, as documented on the provincial healthcare database. A third

advantage of my study is the longer duration of data collection which provided access to medical records for the first 7 years of the children's lives and allowed for the investigation of a longer period of maternal distress in relation to children's cortisol levels than has been reported previously. Lastly, the length of the study allowed for the additional benefit of obtaining a more valid diagnosis of asthma in the children. Asthma diagnoses prior to the age of seven are tentative and, for this reason, many previous studies with younger children report correlations with the occurrence of wheezing and not with actual asthma diagnoses.

Consistent with the previously cited literature, I hypothesized that children with asthma would have a blunted cortisol response to an acute stressor and would exhibit a stronger blunting of the stress response system when exposed to maternal distress in early childhood (during the first year of life). In keeping with the relationship reported by Essex et al. (2002) the strongest blunting effect was expected in children who were exposed to maternal distress both in the first year of life and in subsequent developmental periods (e.g., ages 2-4 years and/or ages 5-7 years). I further hypothesized that non-asthmatic children who were exposed to maternal distress in their first year of life would experience an increased cortisol response to the same acute stressor and that this relationship also would be intensified for children who were exposed to recurrent episodes of maternal distress throughout their childhood.

Method

Participants

A sample of the 1995 Manitoba birth cohort which was originally selected for use in the multidisciplinary, longitudinal project entitled "Study of asthma, genes, and the environment" (SAGE) was used. The SAGE participants were selected from the 16,320 Manitoba births in 1995, of which 13,980 remained living in the province in 2002 (2340 of the birth cohort children were lost to relocation and death). In 2002, brief surveys were mailed to all of the cohort families remaining in Manitoba. The survey consisted of questions pertaining to asthma and allergy symptoms within the families and to the children's home environments (e.g., exposure to pets, mold, and environmental tobacco smoke). From the 3598 completed surveys that were returned, a sample of 723 participants was selected. Included in the final SAGE sample were all of the children who received a diagnosis of asthma by a pediatric allergist (n = 251). An additional 472 participants with no asthma symptoms were recruited to serve as control participants; they were chosen based on a representative stratification of rural and urban residence and SES. Informed consent was obtained from all of the participating families at this and each subsequent stage of the study. All of the families agreed to the use of their healthcare information (complete medical and prescription records) on the provincial healthcare database from 1995 onward. Of the 723 SAGE participants, both maternal distress data and cortisol measures were available for a total of 507 children.

Procedure

Following the initial, brief questionnaire used to recruit participants, a more detailed assessment was conducted. At this time, more comprehensive information regarding respiratory health and the children's home environments was collected. Blood samples were taken as a part of this assessment which also involved the administration of a detailed survey, asthma testing by a pediatric allergist, pulmonary function testing (methacholine challenge), and testing of 16 common allergens (via skin-prick).

Due to the invasive nature of the assessment (withdrawal of the blood sample following the uncomfortable skin pricks for allergy testing), the appointment was considered an acute stressor which provides an indication of the children's stress responsiveness (as measured by their cortisol levels). Blood samples were collected at the end of the assessment appointment, after allergy testing, and an attempt was made to have all of the blood samples drawn as early in the day as possible. It has been suggested that the most reliable method of measuring cortisol levels, to control for intra-individual variation, involves collecting several daily cortisol profiles (multiple daily time samples) and performing a multilevel analysis for each individual participant (Hruschka, Kohrt, & Worthman, 2005). Wust et al. (2000) reasoned, however, that with larger cohort studies, a single measure (the cortisol awakening response) is not only the most practical and inexpensive method of obtaining cortisol levels; it also is sufficiently reliable for epidemiological purposes. The measure obtained here was not the cortisol awakening response but an attempt was made to collect the blood samples during the same time interval (late morning) for all participants, which should assist in controlling for the expected diurnal pattern of cortisol release.

Analyses of serum cortisol levels were conducted in an independent laboratory at the University of Manitoba. The blood samples were diluted 50% with saline and spun over ficoll to obtain white blood cells and plasma, which was immediately frozen. A sample of the plasma was further diluted to a 1:6 ratio, which was then labeled as the 'stock' sample. Finally, the stock sample was diluted to a ratio of 1:240, it was heat denatured at 85° C for 30 minutes, and then it was assayed using a commercially available ELISA kit (Cayman Chemical). The assayed values were measured in ng/ml.

Maternal distress was defined as at least one physician diagnosis of a depressive or anxiety disorder as reported in the participants' medical records or as a record of prescription for antidepressant, anxiolytic, or hypnotic medications in the Manitoba prescription and health care databases. These databases are stored in the Population Health Data Research Repository at the Manitoba Centre for Health Policy. A physician diagnosis of depression or anxiety was indicated by the assignment of an ICD-9 code of 296 (affective psychoses) or 300 (neurotic disorders). Also included were codes 308 (acute reaction to stress), 309 (adjustment reaction), and 311 (depressive disorder, not elsewhere classified). This database measure was expected to be more reliable and valid than a self-report measure, which may be susceptible to recall bias and/or to a positive response bias.

The validity of this database-defined maternal distress measure was tested against self report of postnatal distress in a sample of 454 mothers from the SAGE cohort. It was discovered that the database measure was specific (85%, 95% CI: 78-87%) but not sensitive (21%, 95% CI: 16-27%) to maternal distress experienced during the child's first year of life (Yallop et al., 2006). By using this database definition of maternal distress,

some mothers who experienced distress likely were missed, making the overall results more conservative than what actually occurred. This outcome is considered to be preferable to the possibility of artificially inflated results due to the use of a self-report measure.

The measure of maternal distress was designated as present or not-present during three separate time periods based on the birth of the cohort child: first year of age, ages 2-4 years, and ages 5-7 years. The measure of maternal distress then was classified as no-distress, late distress (occurring during 2-4 or 5-7 years of age), post-natal distress (1st year only), or recurrent distress (present postnatally as well as during at least one other time period). These levels of maternal distress allowed for the investigation of the previously reported results that exposure to maternal distress during the first year of life sensitizes children to later exposure to maternal distress such that recurrent exposure (during a later developmental period) will have a statistically significant effect on cortisol levels (Essex et al., 2002).

Asthma in the cohort children was defined as a diagnosis of asthma made by a pediatric allergist during the assessment appointment when the children were between the ages of 7 and 10 years. The diagnoses were based on the criteria set out by the Canadian Asthma Consensus Guidelines (Becker et al., 2005) and involved a detailed, standardized symptom questionnaire focused on histories of cough and wheeze, allergic conditions, and reactions to asthma medications. A physical examination also was conducted, which centred on chest symptoms such as hyperinflation, prolonged expiration, wheeze, and decreased breath sounds.

A number of possible confounding variables were examined, all of which may have an effect on children's cortisol levels, the dependent measure in my research, as well as on one or both of the independent variables of interest (the presence of asthma and exposure to maternal distress). The potential confounding variables considered were gender, age, time of blood sample, low birth weight, a family history of asthma, the use of corticosteroid medications, early exposure to environmental tobacco smoke, SES, and the duration of exclusive breastfeeding during infancy. The details of all of the variables included in this study are outlined in Table 1.

Age was calculated using the child's date of birth and was recorded in number of years as 7, 8, 9, or 10. Data on confounding factors including a family history of asthma, SES, maternal smoking during the first year of life, and the duration of exclusive breast feeding were collected via the detailed self-report questionnaire during the assessment appointment. Family history of asthma was defined as parental self-report of asthma and was categorized as no family history of asthma, a history of one parent with asthma, or a history of both parents with asthma. Family income was queried and the SES measure was classified as low if the family income was below $20,000 CDN; as average if family income was between $20,000 and $80,000 CDN; and as high if the reported income was above $80,000 CDN. The mothers were asked whether they ever smoked and if they smoked for more than 6 months during the child's first year of life. The measure was dichotomized as exposure or no exposure to maternal ETS during the first year of life. The mothers were asked when formula or cow's milk was introduced into their infant's diet and the measure of exclusive breastfeeding was defined as exclusive if there was no introduction of cow's milk or formula for the first 12 weeks of life and as not exclusive if

Table 1

Independent, Dependent, and Control Variables for Multiple Linear Regression Analysis

Variable			
Type	Name	Definition	Measure
Dependent Variable	Cortisol Level	Serum cortisol level	continuous measure of serum cortisol in ng/ml
Independent Variables	Maternal Distress	Physician diagnosis of depression or anxiety (specified ICD-9 codes) or a prescription for depression or anxiety related medications during three time intervals: first year of life, ages 2-4, & 5-7 years	0 = no distress 1 = late episode only (ages 2-4 or 5-7) 2 = 1st year only (1st year) 3 = chronic distress (1st year plus ages 2-4 and/or 5-7)
	Asthma	Physician diagnosis at age 7-10	0 = no asthma 1 = asthma
Possible Confounding Variables	Gender	Male or Female	0 = male 1 = female
	Age	7, 8, 9, or 10	0 = 7; 1 = 8; 2 = 9; 3 = 10
	Family history of asthma	Reported history of parental asthma	0 = no asthma 1 = one parent 2 = both parents
	SES	Reported level of income	0 = low (< $20k) 1 = moderate ($20k-$80k) 2 = high (>$80k)
	ETS	Reported history of maternal smoking during 1st year of life	0 = mother did not smoke 1 = mother smoked
	Low Birth Weight	Recorded weight at birth, low birth weight is < 2.5 kg	0 = low (< 2.5 kg) 1 = not low (>2.5 kg)
	Exclusive Breastfeeding	Reported duration of exclusive breast feeding	0 = < 12 weeks 1 = > 12 weeks
	Inhaled GC's	Prescription of oral and/or inhaled corticosteroid medication	0 = no 1 = yes

SES = Socio-Economic Status, ETS = Environmental Tobacco Smoke, GC's = Glucocorticoids

they were introduced prior to 12 weeks of age. Information regarding the birth weight of the cohort children was obtained through the provincial healthcare database and the measure of low birth weight was defined as a birth weight under 2.5 kg. The survey included questions regarding the use of common asthma medications. The parents were asked to indicate the type of medication used by their child as well as the frequency of use and this information was corroborated by information in the provincial pharmacare database. The measure of current use of corticosteroid medication was categorized as yes, if either oral or inhaled corticosteroid medication were currently being used, and as no if there was no indication of the use of these types of medications. All of these factors have been found to correlate with cortisol measures and with asthma, but the relationship between exposure to maternal distress during childhood and cortisol levels at ages 7-10 in children with and without asthma is expected to be independent of these potentially confounding variables.

Data Analysis

Preliminary univariate regression analyses were conducted in order to determine the predictability of each of the independent variables for children's cortisol levels when considered in isolation. Following the univariate regression analyses, all of the potential confounding variable variables were entered into a multiple linear regression analysis to determine whether their association with the other variables affected their predictability of cortisol levels. Only those confounding variables demonstrating a statistically significant association with the dependent measure (at the $p < .05$ level) were included as covariates in the final multiple linear regression analysis.

After determining the effects of the potential confounding variables, a multiple linear regression analysis was conducted. The covariates that were identified by the preliminary analyses were entered into the first regression model to examine their combined effects on the dependent measure, cortisol. Along with the covariates, the second model included the two independent variables of interest, namely, maternal distress and asthma. For this analysis, dummy variables were created for the maternal distress variable, a categorical variable, in order to examine the effect of the different levels of maternal distress on cortisol levels, relative to the no distress category. With the presence of a main effect of one of the independent variables of interest confirmed, a third regression analysis was conducted which included the interaction between asthma and maternal distress. Again, due to the categorical nature of the maternal distress variable, dummy variables were created for the interaction variable, allowing for the examination of possible interactions between each level of maternal distress and the presence of asthma. For all of these analyses, a significance level of $p < .05$ was chosen to indicate that the associations found were above chance levels.

Beta weights that were statistically significant at the chosen alpha level of .05 identified which of the variables included in the multiple regression analysis were predictive of children's cortisol levels. The values of the beta weights provide an indication of the magnitude and direction of the association between each independent variable and the dependent variable such that, for each one unit increase in the independent variable (or each factor change for categorical variables), the dependent variable changes by the amount of the beta weight. Whereas the standardized beta weights indicate the independent predictive value of each of the variables, the

unstandardized regression coefficients (B) provide an indication of the predictive value of each of the variables in relation to all of the other variables. These unstandardized regression coefficients were combined to determine the predicted cortisol levels for each interaction between asthma and maternal distress.

The regression analysis was followed by pairwise comparisons in an attempt to determine whether the children's cortisol levels were differentially affected by exposure to maternal distress during different developmental periods. Separate analyses were conducted for children with and without asthma. These pairwise comparisons were made using Tukey's Honestly Significant Differences Test (Tukey's HSD) which controls for the possibility of finding a statistically significant difference between groups simply due to the fact that multiple significance tests are being conducted. Tukey's HSD ensures that the alpha level of the test (in this case, $\alpha = .05$) is preserved and, therefore, that we can have confidence in the finding of a statistically significant difference between groups. A statistical correction was implemented to adjust the alpha level required to indicate statistical significance for each comparison of the multiple comparison test. This was accomplished by dividing the desired alpha level by the number of comparisons (.05/3) which resulted in a cut-off level of $\alpha = .017$ for each comparison. An additional post-hoc analysis was conducted to determine whether there were significant differences in cortisol levels between children with and without asthma at each level of maternal distress.

In a preliminary analysis of the data, it was discovered that cortisol was not normally distributed, but was positively skewed, making it necessary to transform the data to ensure the accuracy and integrity of the statistical tests. A log transformation was

conducted and the resulting distribution was found to be normal. The transformed data were used for all major analyses, but standard measure means are reported to facilitate interpretation of the results. Four outliers were identified and removed from the dataset, one that was more than three standard deviations above the mean and three that were more than three standard deviations below the mean. Removing these extreme cortisol measures from the dataset was important for the reliability of the regression analysis, since outliers can seriously bias results by pulling the regression line in the direction of the extreme scores. The removal of these outliers can be justified conceptually as well, since they may be indicative of an underlying hormone regulation problem (e.g., Addison's disease) and are, therefore, not representative of the population of interest. It also is possible that the extreme values are due to measurement errors. Pollard (1995) reported that measurement errors can occur as a result of the processes involved in collecting, storing, and analyzing cortisol sample and, therefore, it was thought to be equally important to remove these outliers for these potential reasons. Similar procedures are customary in studies using cortisol data (e.g., Ashman, Dawson, Panagiotides, Yamada, & Wilkinson, 2002; Bugental, Marorell, & Barraza, 2003; Azar, 2006).

Results

The final sample consisted of 503 children, 315 with no asthma and 188 children with an asthma diagnosis. Of the non-asthmatic children, 170 (54 %) were male, 145 (46.0 %) were female and the mean age was 8.59 years (SD = .59). In the group of children with asthma, 113 (60.1 %) were male and 75 (39.9 %) were female with an average age of 8.27 years (SD = .60). The groups were distributed similarly across all of the variables included in the study except for the measure of the use of corticosteroid medication. On this measure, only 5.4 % of the non-asthmatic children as compared to 40.4 % of the asthmatic children were using this type of medication. It should be noted that, although corticosteroid medication is commonly prescribed for asthma symptoms, it also is prescribed for treatment of other inflammatory conditions (e.g., oral corticosteroids for dermatitis and allergic reactions and inhaled corticosteroids for virus associated wheezing), which may account for the use of the medication in the non-asthmatic children. Table 2 summarizes the demographic and other characteristics of the participants.

With respect to the potential confounding variables included in this study, the results of a preliminary regression analysis indicated that only low birth weight and age were predictive of the children's cortisol levels. For low birth weight, $\beta = .110$, $t(501) = 2.481$, $p = .013$, and the proportion of variance accounted for by low birth weight also was statistically significant with $R^2 = .012$, $F(1,501) = 6.156$, $p = .013$. The positive association indicates that children born with a low birth weight (under 2.5 kg) had higher cortisol levels than children who were born with a healthy birth weight. For the age variable, an increase in age was associated with an increase in cortisol levels, $\beta = .116$,

Table 2

Distribution of Population Characteristics

		No Asthma	Asthma
Total Participants		315	188
Gender	Male, n (%)	170 (54.0)	113 (60.1)
	Female, n (%)	145 (46.0)	75 (39.9)
Age (years)	7, n (%)	7 (2.2)	13 (6.9)
	8, n (%)	124 (39.4)	114 (60.6)
	9, n (%)	174 (55.2)	59 (31.4)
	10, n (%)	10 (3.2)	2 (1.1)
	Mean ± SD	8.59 ± .59	8.27 ± .60
Maternal Distress	None, n (%)	127 (40.3)	70 (37.2)
	Late Only, n (%)	134 (42.5)	87 (46.3)
	1st Year Only, n(%)	11 (3.5)	6 (3.2)
	Recurrent/Chronic, n (%)	43 (13.7)	25 (13.3)
Low Birth Weight (< 2.5 kg)	Yes, n (%)	15 (4.8)	13 (6.9)
	No, n (%)	300 (95.2)	175 (93.1)
Time of Serum Sample	Mean ± SD	12:26 ± 2:16	11:41 ± 1:54
Corticosteroid Medication	Yes, n (%)	17 (5.4)	76 (40.4)
	No, n (%)	298 (94.6)	112 (59.6)
Family History of Asthma	None, n (%)	247 (78.4)	130 (69.1)
	One Parent, n (%)	61 (19.4)	52 (27.7)
	Both Parents, n (%)	7 (2.2)	6 (3.2)
Socioeconomic Status	Low, n (%)	9 (2.9)	10 (5.3)
	Middle, n (%)	192 (61.0)	106 (56.4)
	High, n (%)	88 (27.9)	63 (33.5)
	Missing, n (%)	26 (8.3)	9 (4.8)
Exclusively Breastfed	Yes, n (%)	110 (34.9)	88 (46.8)
	No, n (%)	194 (61.6)	93 (49.5)
	Missing, n (%)	11 (3.5)	7 (3.7)
Maternal Smoking (1st year)	Yes, n (%)	39 (12.4)	43 (22.9)
	No, n (%)	269 (85.4)	142 (75.5)
	Missing, n (%)	7 (2.2)	3 (1.6)

of the potential confounding variables considered. Specifically, gender, time of serum sample collection, use of corticosteroid medication, family history of asthma, SES, maternal smoking during the first year of life, and duration of exclusive breastfeeding in infancy were not found to be predictive of cortisol levels. These same potential confounds were entered into a multiple regression analysis to examine the predictability of each variable while taking into consideration their associations with the other variables. It was discovered that low birth weight maintained its significant predictability of cortisol levels ($\beta = .131$, $t(445) = 2.725$, $p = .007$). Age also remained significantly predictive of cortisol levels with $\beta = .130$, $t(445) = 2.606$, $p = .009$. None of the other potential confounding variables produced statistically significant results. Due to the combination of these outcomes, birth weight status and age were included as covariates for all further analyses. Table 3 shows the results of these preliminary analyses.

Asthma diagnosis and maternal distress also were analyzed in isolation of the other variables. Mean cortisol levels were found to be lower in the children with asthma (47.45, SD = 26.47) than in the children with no asthma (M = 51.71, SD = 32.68), but this difference was not found to be statistically significant ($\beta = -.044$, $t(501) = -0.977$, $p = .329$). Therefore, asthma diagnosis, when considered alone, did not reliably predict cortisol levels for these children. In contrast, cortisol levels were significantly predicted by the level of exposure to maternal distress, $\beta = .104$, $t(501) = 2.340$, $p = .020$. The highest mean values were found in children exposed to maternal distress during the first year of life only (M = 73.52, SD = 38.76) followed by those exposed to recurrent maternal distress, including the post-natal period (M = 52.64, SD = 30.55). Lower mean cortisol levels were found in children who were never exposed to maternal distress (M =

Table 3

Predictability of Children's Cortisol Levels for All Potential Confounding Variables (in Isolation and in Combination With All Other Variables)

		Univariate		Multivariate	
	n	β	***p***	β	***p***
Age	503	.116	.009	.130	.009
Low Birth Weight	503	.110	.013	.131	.007
Gender	503	-.022	.629	-.007	.880
Corticosteroid Medication	503	-.057	.199	-.025	.608
Family History of Asthma	503	-.033	.456	-.040	.405
Sample Time	500	.005	.909	-.031	.527
Maternal Smoking	493	.001	.989	-.005	.923
Exclusive Breastfeeding	485	.023	.613	.031	.515
SES	468	-.019	.689	-.011	.811

46.48, SD = 27.97) and in those who were exposed to maternal distress commencing after their first year of life (M = 50.78, SD = 31.34). Maternal distress, when considered in isolation of all of the other variables, accounted for a significant proportion of variance in cortisol levels, $R^2 = .011$, $F(1,501) = 5.475$, $p = .020$.

The final regression analysis, a multiple linear regression analysis, consisted of three steps. The first step analyzed the two covariates that were determined by the preliminary analysis (low birth weight status and age), the second step included the two main independent variables of asthma diagnosis and level of exposure to maternal distress in an attempt to identify whether there were any main effects for these variables, and the third step involved the addition of the interaction variable (asthma diagnosis by maternal distress). The results of this analysis can be seen in Table 4.

In the first model, the proportion of variance accounted for in the prediction of cortisol levels was statistically significant, $R^2 = .026$, $F(2,500) = 6.779$, p = .001. Low birth weight significantly predicted cortisol levels $\beta = .114$, $t(500) = 2.579$, $p = .01$ which indicates that children who were born with a low birth weight have reliably higher cortisol levels. Age also was a statistically significant predictor of cortisol with $\beta = .119$, $t(500) = 2.706$, $p = .007$. This relationship indicates a rise in cortisol levels with increasing age. Following this analysis, maternal distress and asthma diagnosis were entered into the model.

The second model, which included the two main independent variables of interest, accounted for a higher proportion of variance in the prediction of cortisol levels, $R^2 = .047$, $F(6,496) = 4.080$, p = .001. Low birth weight continued to be a significant predictor of cortisol levels, $\beta = .095$, $t(496) = 2.147$, $p = .032$, as did age, $\beta = .107$, $t(496)$

= 2.348, p =.019. Maternal distress during the first year of life was found to significantly predict cortisol levels, β = .138, t(496) = 3.032, p =.003, while asthma diagnosis was not, β = -.021, t(496) = -.464, p =.643. Although the main effect of asthma was not found to be statistically significant, the relationship was in the expected direction in that cortisol levels were lower in children with a diagnosis of asthma.

With a statistically significant main effect of maternal distress and the effect of asthma diagnosis in the expected direction, a third model was tested which included the interaction between maternal distress and asthma. With all of the predictors entered into the model, the proportion of variance accounted for was higher than in the two previous models and remained significant with R^2 = .059, F(9,493) = 3.444, p = .000. Low birth weight and age retained their significant associations with cortisol, resulting in values of β = .096, t(493) = 2.176, p =.030 and β = .110, t(493) = 2.420, p =.016, respectively. With the interaction between maternal distress and asthma entered into the model, postnatal maternal distress retained its significant predictability, β = .152, t(493) = 2.699, p =.007 and recurrent/chronic distress also was significantly predictive of cortisol levels, β = .163, t(493) = 2.760, p =.006. In this model, asthma diagnosis alone was still not a statistically significant predictor of cortisol levels. The interaction between asthma diagnosis and maternal distress, however, was found to be a significant predictor of children's cortisol levels in that exposure to recurrent or chronic maternal distress, beginning in the first year of life, for children with asthma was predictive of lower cortisol levels β = -.157, t(493) = -2.518, p =.012.

Table 4

Summary of Multiple Linear Regression Analysis for Variables Predicting Cortisol Levels in Children Aged 7 – 10 Years (N = 503)

Variable	R^2	F	B*	B**	95% Confidence Interval for β	p
Model 1	.026	6.779				.001
Age			.106	.119	.029 to .182	.007
Low Birth Weight			.269	.114	.064 to .474	.010
Model 2	.047	4.080				.001
Age			.095	.107	.015 to.174	.019
Low Birth Weight			.225	.095	.019 to.432	.032
Asthma Diagnosis			-.024	-.021	-.124 to .076	.643
Maternal Distress						
Late Only			.069	.063	-.034 to .172	.189
Postnatal Only			.413	.138	.145 to .681	.003
Recurrent/Chronic			.116	.073	-.031 to .264	.122
Model 3	.059	3.444				.000
Age			.097	.110	.018 to .176	.016
Low Birth Weight			.228	.096	.022 to .434	.030
Asthma Diagnosis			.080	.071	-.079 to .238	.325
Maternal Distress						
Late Only			.105	.096	-.024 to .234	.112
Postnatal Only			.455	.152	.124 to .787	.007
Recurrent/Chronic			.259	.163	.074 to .443	.006
Asthma x Maternal Distress Interaction						
Asthma x Late Only			-.102	-.071	-.314 to .110	.345
Asthma x Postnatal			-.122	-.024	-.674 to .431	.666
Asthma x Recurrent			-.391	-.157	-.696 to -.086	.012

* = Unstandardized coefficient; ** Standardized coefficient

Using the unstandardized regression coefficients, it was discovered that, in children with asthma, when compared to children who were never exposed to maternal distress, exposure only to late distress predicted an 8.3 % increase (not significant) in cortisol levels while exposure only during the first year of life predicted a 41.3 % increase (significant) in cortisol levels and recurrent or chronic exposure predicted a 5.2% decrease (significant) in cortisol levels. In children with no asthma, late distress predicted a 10.5% increase (not significant), postnatal distress predicted a 45.5% increase (significant), and recurrent or chronic distress predicted a 25.9% increase (significant) in cortisol levels over the levels of children who were never exposed to maternal distress. The calculation of these values can be found in Table 5. The next analyses involve pairwise comparisons of the obtained means to determine which group differences were statistically significant.

The first pairwise comparisons were conducted to examine the differences in cortisol levels among the separate maternal distress categories. Figure 1 shows the group means both for children with and without asthma. As with the multiple linear regression analysis, results were adjusted for low birth weight and age. Figure 2 shows the comparison of the adjusted means. Mean cortisol levels for children with asthma at each level of maternal distress were as follows: no distress, M = 45.79 (SD = 21.53), late distress only, M = 49.18 (SD = 30.46), postnatal distress (1^{st} year) only, M = 68.79 (SD = 35.95), and recurrent/chronic distress, M = 40.95 (SD = 18.91). Upon testing for pairwise comparisons, no statistically significant differences were found between any of these groups.

Table 5

Calculation of Predicted Change in Cortisol Values Using Unstandardized Regression Coefficients

Measure	Asthma	Distress	Interaction	Predicted Change[†]
Asthma x Late Distress Only	.08	+ .105	- .102	= .083(100) = 8.3%
Asthma x Postnatal Distress Only	.08	+ .455	- .122	= .413(100) = 41.3%*
Asthma x Recurrent/Chronic Distress	.08	+ .259	- .391	= -.052(100) = - 5.2%*
No Asthma x Late Distress Only	0	+ .105	+ 0	= .105(100) = 10.5%
No Asthma x Postnatal Distress Only	0	+ .455	+ 0	= .455(100) = 45.5%*
No Asthma x Recurrent Distress	0	+ .259	+ 0	= .259(100) = 25.9%*

[†]Change relative to children with no exposure to maternal distress
*Significant at $p < .05$

In children with no asthma, the mean cortisol level for the group that was never exposed to maternal distress was 46.86 ng/ml (SD = 31.03), which did not significantly differ from the group of children that were exposed to maternal distress only after the first year of life (M = 51.82, SD = 31.97). Cortisol levels were higher in non-asthmatic children who were exposed to postnatal maternal distress during the first year of life only (M = 76.10, SD = 41.67) than they were in children who were never exposed to maternal distress. Following a Bonferroni correction procedure, this difference was found to be statistically significant at the $p < .05$ level. Non-asthmatic children exposed to recurrent maternal distress had a mean cortisol level of 59.43 ng/ml (SD = 33.99) which was higher than non-asthmatic children who had never been exposed to maternal distress, but this difference was not found to be statistically significant at the $p < .05$ level following the Bonferoni correction. No other statistically significant group differences were found.

The final analysis involved a comparison of mean cortisol levels for children with and without asthma at each level of maternal distress, again controlling for the effects of low birth weight and age. The following results are consistent with the outcome of the multiple regression analysis in terms of the predicted cortisol levels for each asthma by maternal distress interaction. For children who had never been exposed to maternal distress, cortisol levels for both diagnostic groups did not significantly differ. Those with asthma (n = 70) had a mean cortisol value of 45.79 (SD = 21.53) and those without asthma (n = 127) had a mean of 46.86 (SD = 31.03). A similar comparison of means was found in children who had been exposed to maternal distress only after their first year of life with asthmatic children (n = 87) generating a mean cortisol value of 49.18 (SD = 30.46) and non-asthmatic children (n = 134) displaying a mean of 51.82 (SD = 31.97).

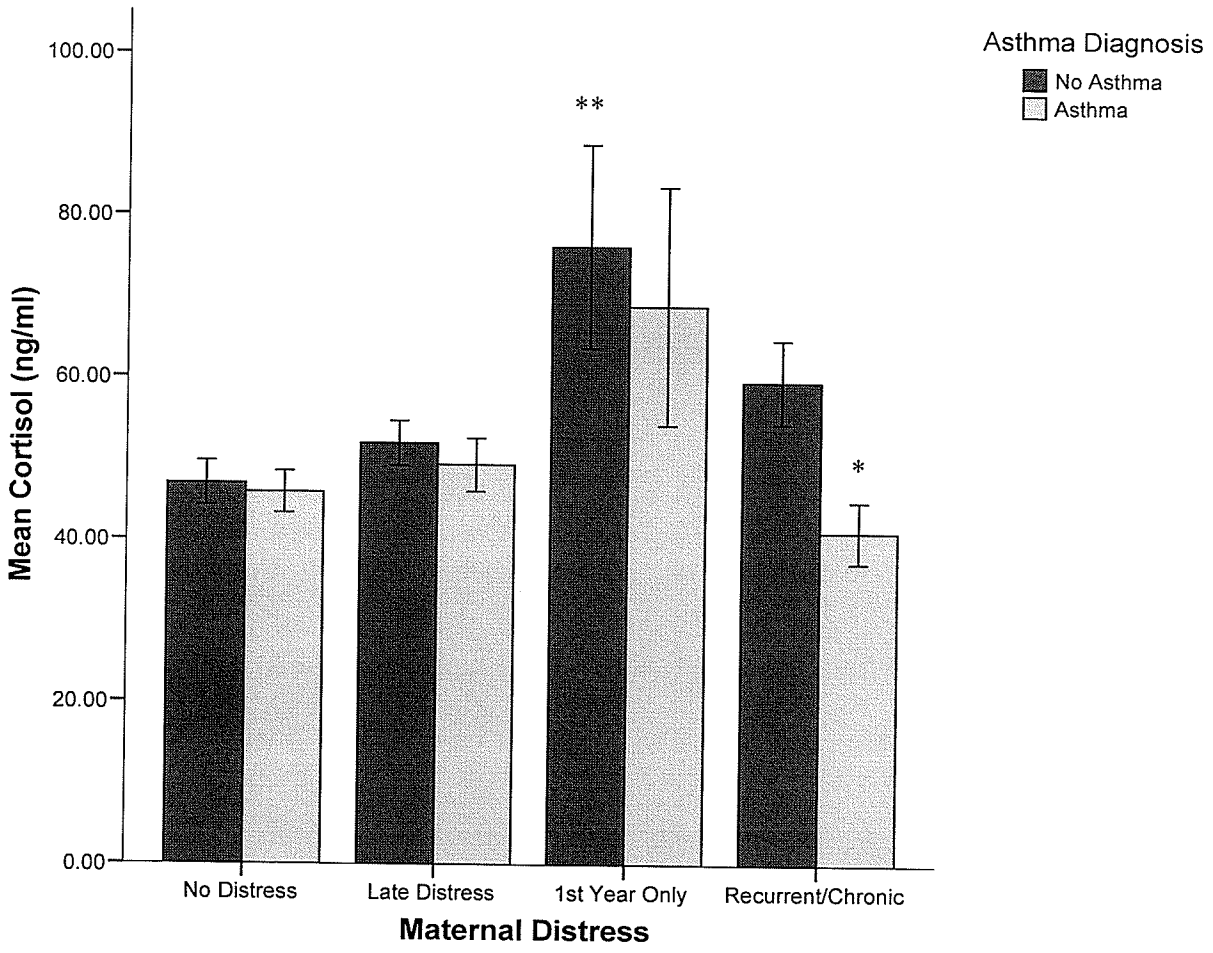

Figure 1. Cortisol levels (Raw score mean ± SEM) in response to an acute stressor for children with and without asthma (7 – 10 years old) exposed to different levels of maternal distress throughout childhood. * Difference from no-asthma group with recurrent/chronic exposure, significant at $p < .05$. ** Difference from no-asthma, no distress group, significant at $p < .05$.

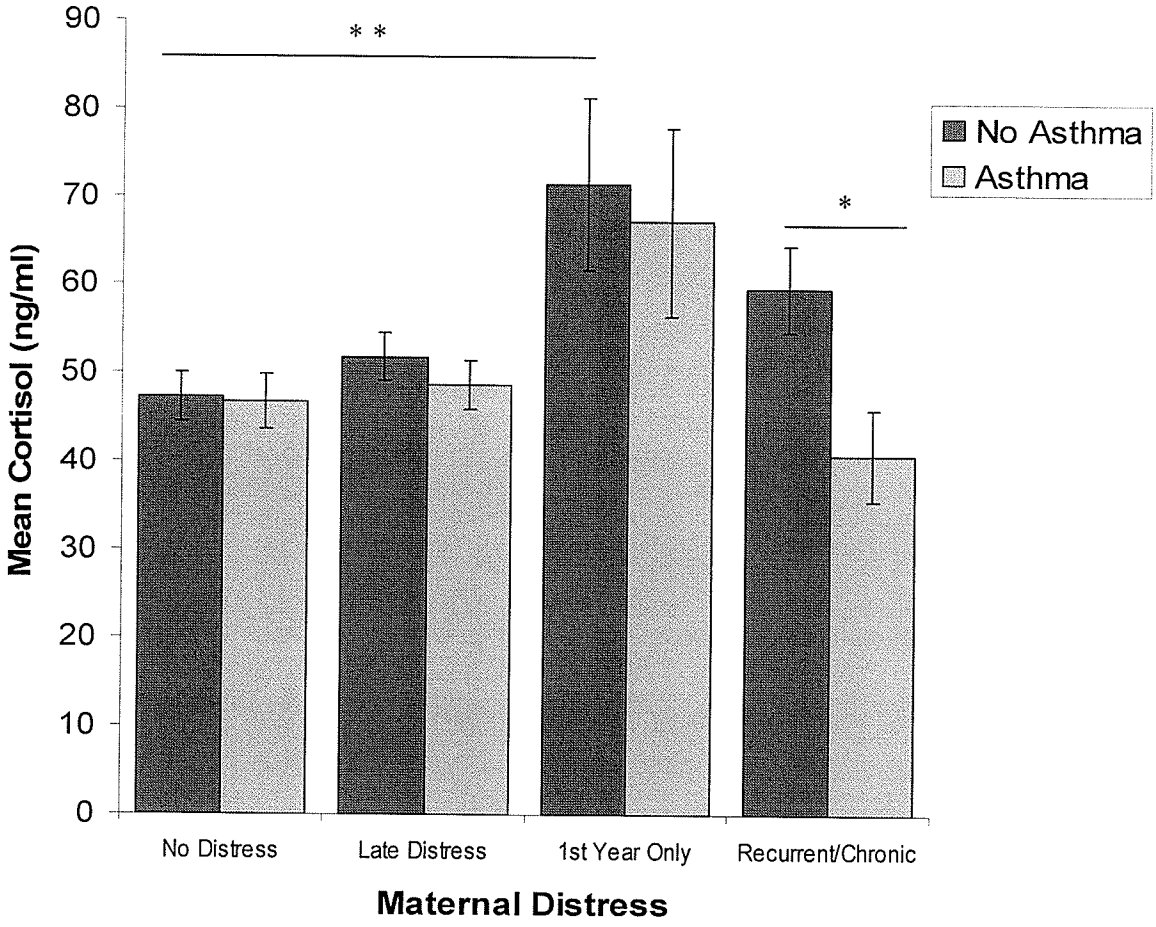

Figure 2. Cortisol levels, adjusted for age and low birth weight (adjusted mean ± SEM), in response to an acute stressor for children with and without asthma (7 – 10 years old) exposed to different levels of maternal distress throughout childhood. * Difference from no-asthma group with recurrent/chronic exposure, significant at $p < .05$. ** Difference from no-asthma group with no exposure, significant at $p < .05$.

For children who were exposed to maternal distress during the first year of life only (short term postnatal distress), those with asthma (n = 6) had a mean cortisol value of 68.79 (SD = 35.95) and those with no asthma (n = 11) had a mean value of 76.10 (SD = 41.67). The mean difference in cortisol levels, although substantial, was not statistically significant once adjustments were made for age and low birth weight. These unexpected results may be due to the very small number of children in this maternal distress group. Children with asthma who were exposed to maternal distress during the postnatal period as well as later in childhood had a mean cortisol level of 40.95 (SD = 18.91; n = 25) that was significantly lower than children without asthma (M = 59.43, SD = 33.99; n = 43). The results of this analysis can be seen in Table 6, in which adjusted means are reported along with the raw score means.

Table 6

Pairwise Comparisons of Mean Cortisol Levels by Asthma Diagnosis at Each Level of Maternal Distress

	Raw Score Mean (ng/ml) ± SD		Adjusted Mean* (ng/ml) ± SD			
Maternal Distress	No Asthma	Asthma	No Asthma*	Asthma*	Difference*	p
None	45.79 ± 21.53	46.86 ± 31.03	47.29 ± 2.83	46.79 ± 3.11	0.05 ± 4.27	.472
Late Only	51.82 ± 31.97	49.18 ± 30.46	51.84 ± 2.76	48.57 ± 2.78	3.27 ± 4.41	.534
1st Year Only	76.10 ± 41.67	68.79 ± 35.95	71.37 ± 9.81	67.07 ± 10.56	4.30 ± 20.30	.961
Recurrent/Chronic	59.43 ± 33.99	40.95 ± 18.91	59.16 ± 4.58	41.42 ± 6.06	17.75 ± 7.71	.025

*Adjusted for Age and Low Birth Weight Status

Discussion

In a sample of over 500 schoolchildren, I found evidence of a heightened cortisol response in non-asthmatic children exposed to maternal distress during early life. In contrast, however, the cortisol response appeared to be dampened in children with asthma. My analyses were controlled for two important confounding factors, age and low birth weight (Hack, et al, 2005; Rasmalen et al, 2005; Wust et al., 2000). Thus, the effects of maternal distress on cortisol levels in children with and without asthma were independent of these factors. The long-term consequences of maternal distress in early life on children's cortisol levels has been reported by others (e.g., Essex et al., 2002; Halligan et al., 2004; Lupien, King, Meaney, & McEwan, 2000), but my research appears to be the first to assess the influence of a diagnosis of childhood asthma on this association. Furthermore, I used an objective measure of maternal distress over an extended time period, which allowed for the investigation of the effects of maternal distress of a longer duration.

My first hypothesis, that children with asthma who were exposed to repeated episodes of maternal distress beginning in infancy would have blunted cortisol levels in response to an acute stressor, was based mainly on the findings of Buske-Kirschbaum et al. (2003) and of Essex et al. (2000). Buske-Kirschbaum et al. (2003) reported that children with asthma have a blunted cortisol response to acute, psychological stressors in comparison to healthy controls while Essex et al. (2000) found significantly altered cortisol levels in children who were exposed to recurrent postnatal distress. The formulation of my hypothesis also was influenced by the reports of other researchers who found correlations between exposure to maternal distress and increased asthma symptoms

(e.g., in children, Horwood, Fergusson, & Shannon, 1985 and Klinnert et al., 2001; in animals, Chida et al., 2007). The finding that previous exposure to persistent maternal distress combined with a diagnosis of asthma predicted lower cortisol levels confirmed my hypothesis. The discovery of a difference in cortisol levels in children with and without asthma who were exposed to recurrent maternal distress beginning in infancy, whereby asthmatic children responded with significantly blunted cortisol levels in comparison to non-asthmatic children, supported my hypothesis of an interaction between the two independent variables of interest. The finding that children with and without asthma exhibited similar cortisol responses when they had never been exposed to maternal distress, when they had been exposed to the distress only after their first year of life, and when they were exposed to it only during the first year of life indicates that the timing and degree of the exposure to maternal distress is an important factor in the interaction. The discovery that asthmatic children with a history of recurrent exposure to maternal distress respond to an acute stressor with blunted cortisol levels as compared to non-asthmatic children is consistent with the previous finding of an attenuated cortisol response in children with asthma (Buske-Kirschbaum et al., 2003), implicates previous exposure to maternal distress in the effect.

With respect to the children with no asthma, my hypothesis that these children would respond to an acute stressor with an elevated cortisol response if they had been exposed to recurrent maternal distress beginning in the postnatal period also was supported. My results for the non-asthmatic children were consistent with the findings of Essex et al. (2002), who reported significantly elevated cortisol levels in children who were exposed to recurrent maternal distress and no elevation in cortisol levels in children

who were never exposed to maternal distress or in those who were exposed to distress only after the first year of life (at age 4.5 years in the study by Essex et al, 2002). My findings for the group of children who were exposed to maternal distress exclusively during their first year of life differ from Essex et al. (2002) in that my study revealed a statistically significant elevation in cortisol levels whereas Essex and colleagues did not. This difference may be due to my use of a database definition (record of physician diagnosis and prescription history) for the maternal distress measure, which may have identified only the most severe cases of postnatal maternal distress. Despite this difference, my study supports the findings of Essex et al. (2002), that exposure to recurrent maternal distress beginning in the first year of life significantly alters cortisol levels, and that this effect on children's stress response systems extends beyond the preschool age (4.5 years old) to the age of 10 years.

In combination, these findings suggest that, as hypothesized, the stress response systems of children with asthma are affected differently by exposure to recurrent maternal distress than their healthy counterparts. Essex, et al. (2002) concluded that early exposure to maternal distress may sensitize children to subsequent stress exposure. My results support this conclusion, with the additional qualification that for children with no asthma, there is a heightened cortisol response to an acute stressor whereas, for children with asthma, the effect is an attenuation of cortisol secretion in response to the same stressor.

Study Limitations

One limitation of my study is its retrospective nature which limited the data that were available and did not allow for the collection of any additional information.

Without having cortisol measures from the children's earlier stages of development, information regarding the onset of the altered stress response was not available. Without this information, it is not possible to determine whether the blunted stress response was present prior to the diagnosis of asthma or if it occurred following the asthma diagnosis. Without an earlier measure of stress response functioning, I could not establish whether the altered cortisol response is an early indicator of asthma, possibly playing a causal role in the onset of asthma as suggested by Ball et al. (2005), Buske-Kirschbaum et al. (2003), and Landstra et al. (2002) or whether the change in the stress response is a symptom of asthma or, alternatively, is the result of dealing with the additional stressor of experiencing the symptoms of a chronic disease. Also, as the SAGE cohort was originally selected to study the effects of genetic and environmental factors on the development of asthma symptoms, the data that were collected were more appropriate to factors associated with asthma than to those factors that are associated with changes in cortisol levels. Some factors that are known to affect cortisol levels were included in the study (e.g., age, gender, SES, asthma medications, and low birth weight), but others would have been similarly informative and would have helped to statistically control for the variability among the scores. Such variables as maternal prenatal factors (smoking and distress), baseline cortisol levels, perceived distress, current state of health, sleep and activity patterns, and smoking, napping, and the consumption of food or beverages prior to cortisol measurement all may have affected the cortisol levels obtained in my research. Such factors may have contributed to the substantial variability observed and are likely responsible for the low explained variance that was found. It is possible that measurement error accounted for some of the variance, but Kirschbaum et al. (1990)

stated that when measurement error does occur, the variance attributable to it accounts for only a small proportion of total variation. Therefore, even though it is clear that the final regression model, which included the covariates, the main effects and the interaction effects, had the highest explained variance (which is desirable) and resulted in a statistically significant association (at the $p < .0001$ level), the extremely low explained variance indicates that any conclusions from these results should be regarded with some caution.

A second, but related, limitation was the absence of a clear measure of the perceived stressfulness of the assessment appointment, which was identified in the study as an acute stressor. It is possible that some children found the appointment extremely stressful, while others found it only mildly stressful, and still others may not have found it stressful at all. These differences in perceived stress may be reflected in the different cortisol levels obtained. It seems plausible that children with asthma may be more accustomed to invasive procedures during medical appointments and thus, may not have found the assessment as stressful as the children without extensive exposure to medical appointments. As discovered by Dickerson and Kemeny (2004) through their meta-analysis concerned with the effects of acute stressors on cortisol responses, tasks involving both uncontrollable and social-evaluative components are associated with the largest changes in cortisol. Accordingly, the use of a standardized psychosocial stress test such as the Trier Social Stress Test (TSST) would have provided more certainty that the acute stressor was perceived as stressful by the children. At the very least, some indication of perceived stress by each of the children would have been useful information.

Another suggested alteration to the data collection procedure is to have collected baseline measures of cortisol prior to the presentation of the acute stressor. This would have provided a better sense of the stress response, as baseline levels could be taken into account. Baseline levels would provide additional information about the general functioning of the HPA axis in the children, as well. Again, if a standardized test such as the TSST were used, collection of baseline and post-stress measures would have been simple to obtain. Alternatively, baseline measures could have been obtained through home collection of saliva samples as used in many studies examining the functioning of the HPA axis (e.g., Essex et al., 2002).

Future Directions

My findings provide preliminary evidence of an interaction between exposure to maternal distress and asthma diagnosis, as they relate to children's cortisol levels in response to an acute stressor. Future studies should include baseline measures of cortisol levels, involving several daily measures to give an indication of diurnal variation, and should make use of a standardized measure of an acute stressor such as the TSST. Equally important would be the control of all known variables related to cortisol measures and to asthma or maternal distress including, but not limited to, age, gender, birth weight, socio-economic status, smoking status (self and family members), family history of asthma, duration of breastfeeding as an infant, illness status (anything from cold/flu to hormone dysregulation problems), current use of medication (any that may affect cortisol levels), time of cortisol collection and a history of sleep patterns (including naps), activity patterns, reported stress level, and food and beverage consumption prior to the sample. In addition, a measure of prenatal distress is recommended for future studies,

as there is evidence of an association between maternal prenatal distress and maternal prenatal cortisol levels with children's cortisol levels in response to acute stressors (Gutteling, B.M., Weerth, C.D., & Buitalaar, J.K., 2004 and 2005; Yehuda et al., 2005).

My findings point to an underlying relationship between the immune system and the stress response system in children with asthma, however, having used regression analyses, it was not possible to determine whether exposure to maternal distress and its subsequent effect on the stress response system was responsible for the development of asthma as hypothesized by Wright et al. (2002) and Chrousos (1997). Also it was not possible to determine if children who will go on to develop asthma are more susceptible to the effects of maternal distress on their stress response systems. A prospective, longitudinal study may provide a better idea of the sequence of events and may help to determine which of these possibilities is more likely.

Alternatively some of the children who did not have a diagnosis of asthma at the time of data collection may go on to be diagnosed with asthma at a later time. If these children are followed though successive developmental periods, it may be possible to determine whether an altered cortisol response was present prior to the onset of asthma symptoms by comparing their cortisol scores at age 7 – 10 years to those at a later age. The results would provide us with a better understanding of the mechanisms involved in the development of asthma, including the role of maternal distress.

Policy Relevance

The finding that the functioning of children's stress response systems differ in children with and without asthma who are exposed to recurrent maternal distress offers additional support for the relationships discovered by Essex et al. (2002), that exposure to

postnatal maternal distress sensitizes children to the effects of later stress exposure. In addition, it corroborates the findings of Buske-Kirschbaum et al. (2003), that children with asthma have an attenuated response to an acute stressor. This newly discovered association, if supported by future research, may have implications for the establishment of health policies related to maternal distress. Programs established based on these policies might be particularly important for helping children with a genetic predisposition to developing asthma but would ultimately be helpful for all children.

One such policy may involve mandatory screening for stress related disorders such as depression and anxiety in new mothers. Manitoba's Families First program is an existing program designed to assess these postpartum disorders. If mothers' stress-related psychological conditions are diagnosed early, treatment can be offered which may help to lessen or even prevent the related negative health effects on children. With treatment, mothers may be better able to provide a healthier, more supportive environment for their children. For example they may be more likely to abstain from smoking (Groer & Morgan, 2007; Lasser et al., 2000) and may breastfeed longer which creates stronger mother-infant bonds that offer protective immune effects and support the development of a healthy stress response system (Tarullo & Gunnar, 2006). These, and similar, improvements might help to alleviate such health risks as the development of asthma and the dysregulation of the stress response system that have been shown to accompany exposure to maternal distress beginning in infancy.

If maternal distress is left undiagnosed and/or untreated, children may be at risk for developing an altered HPA response. Risks are for a possible hyporesponsive system in children with asthma and for a possible hyperresponsive system for children with no

asthma. Both of these altered responses to stressors carry their own troubling health implications. Elevated cortisol in response to an acute stressor, as seen in the children with no asthma, has been correlated with later psychiatric problems such as anxiety and depression (eg. Halligan, Hebert, Goodyer, & Murray, 2006) while a blunted cortisol response has been associated with negative health effects due to an increased risk for the development of stress-related disorders including immune disorders, depression, reproductive problems, chronic fatigue and chronic pain conditions (Heim, Ehlert, & Hellhammer, 2000; Sternberg & Licinio, 2002). Early diagnosis and treatment of maternal distress may not only serve to lessen the risk of an altered stress response in children, but it also may serve to prevent future episodes of distress for mothers which could further alleviate the associated risk to children's health.

In conclusion, the main finding of my research is that exposure to maternal distress in childhood is associated with different cortisol responses to an acute stressor depending upon asthma status. Children with no asthma who have been exposed to postnatal maternal distress respond with significantly elevated cortisol levels in the presence of an acute stressor whereas children with asthma respond with a blunted cortisol response to persistent maternal distress. If further research supports this relationship between maternal distress and the stress response for children with and without asthma, it may be wise to consider the negative impact that this has on children's health. With the rates of asthma and maternal distress both increasing in the Canadian population, a growing number of children are potentially at risk for a number of health complications. A simple precaution such as implementing routine screening for maternal distress during postnatal medical visits could lead to effective treatment for distressed

mothers, which may help to prevent some cases of childhood asthma and may alleviate the symptoms for others. It also may serve to improve HPA axis functioning in children with and without asthma. Such a screening program exists in Manitoba's Families First, which may be found to facilitate these health improvements in children and may concurrently alleviate some of the burden on the healthcare system that is caused by asthma and/or an altered stress response system.

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