DOES PERCEIVED STRESS IN PREGNANT IMMIGRANT WOMEN PREDISPOSE THEIR INFANTS TO ALLERGIC DISEASE DEVELOPMENT?

by

Michal Peer

A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy

Institute of Medical Science
University of Toronto

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Abstract

Prenatal maternal stress (PNMS) is linked with a multitude of negative outcomes in the exposed offspring, including allergic disease development. Pregnant Canadian immigrant women often experience high levels of stress; however, psychosocial contributors to PNMS and their physiologic sequelae remain understudied in this group. This pilot study prospectively examined whether PNMS in Canadian immigrant women was associated with allergic outcomes in their infants, focusing on potential mediators and underlying mechanisms. Seventy-eight women were examined in early-/mid-pregnancy, in late pregnancy, and at a minimum of one year postpartum. Assessments included questionnaires concerning PNMS and other psychosocial determinants of health, collection of saliva samples throughout the day for measurement of cortisol activity, collection of blood samples for measurement of serum cytokine levels, and administration of a skin-prick test on mothers and infants for measurement of allergic sensitization. Low levels of perceived stress in early-/mid-pregnancy were associated with a greater likelihood of allergic sensitization in infants, but this association was mediated by a lack of financial hardship (i.e., high socioeconomic status). Financial hardship and a greater number of
prenatal stressful life events were associated with lowered cortisol activity in early-/mid-pregnancy. A trend was also seen for higher levels of IL-10 during late pregnancy in women reporting financial hardship. These results are limited by a small sample size that further decreased at each subsequent study (i.e., significant participant attrition).

Nevertheless, the novelty of this study and the numerous strengths in its design (including women with a language barrier, objectively measuring atopy in mothers and infants, and examining underlying mechanisms) make this a unique contribution to the literature. Furthermore key facilitators and barriers to conducting health research in this group were identified that may aid future studies aiming to test these preliminary results in a larger sample size.
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It is not an easy task to put into words the tremendous support and guidance I have received over the last decade (plus) from my supervisor and mentor, Dr. Meir Steiner. I am overwhelmed by the generosity he has displayed toward me from our first point of contact until today. Since the beginning, I felt privileged to be in his presence, to learn from him, and to be associated with him and his work – and I feel that way today, even more so. Dr. Steiner holds extremely high standards for his research and his students; this challenged, but also motivated me through every step of my graduate career. He is an extraordinarily hard-working man who expects the same of his colleagues; the result is that all those around him rise to a higher level. Additionally, Dr. Steiner was always there for me, no matter how many other things he was juggling. He is a kind, caring man who also considered my well-being over and above what might be expected of a graduate supervisor and for all this, and more, I am eternally grateful. Despite all this, or perhaps because of it, I still can’t bring myself to call him Meir. Dr. Steiner, thank you for everything you’ve done for me.

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This research would not have been possible without my team at the Firestone Research Institute, spear-headed by Dr. Parameswaran Nair. Dr. Nair was supportive and kind beyond words and it is difficult to quantify how much that means to me. Particular thanks also go to Melanie Kjarsgaard and Shauna Denis, who conducted the allergy testing and
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Contributions

This thesis was prepared solely by the author, M. Peer. All the work presented herein was conducted, in whole or in part, by M. Peer with the exception of some technical aspects, specifically:

1) Skin-prick testing of mothers and infants, performed by Melanie Kjarsgaard, Shauna Denis, and Dennis Kamada

2) Serum cytokine assay, performed by Marg Coote
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List of Abbreviations

11βHSD-2 – 11β hydroxysteroid dehydrogenase 2
ACTH – adrenocorticotropic hormone
ANCOVA – analysis of covariance
ANS – autonomic nervous system
APC – antigen-presenting cell
B cell – bone-marrow derived cell
BMI – body mass index
CBR – community-based researchers
CNS – central nervous system
CRH – corticotrophin-releasing hormone
CRISYS-R – Crisis in Family Systems – Revised
CRISYS-R-neg – CRYSIS-R negative score
DSM-IV – Diagnostic and Statistical Manual of Mental Disorders
ELISA – enzyme linked immunoassay
EPDS – Edinburgh Prenatal Depression Scale
GA – gestational age
GC – glucocorticoid
GCR – glucocorticoid receptor
HPA axis – hypothalamic-pituitary-adrenal axis
HTQ – Harvard Trauma Questionnaire
IFN-γ - interferon-γ
IgE – immunoglobulin E
IL – interleukin
LBW – low birth weight
LPS – lipopolysaccharide
mRNA – messenger ribonucleic acid
MSPSS – Multidimensional Scale of Perceived Social Support
NK cell – natural killer cell
PNMS – prenatal maternal stress
PNS – peripheral nervous system
PPD – postpartum depression
PRIME-MD PHQ - Primary Care Evaluation of Mental Disorders Patient Health Questionnaire
PSS – Perceived Stress Scale
PTB – preterm birth
PTSD – post-traumatic stress disorder
RS – Resiliency Scale
SAM – sympathetic adrenal-medullary
SD – standard deviation
SES – socioeconomic status
SLE – stressful life events
SPT – skin prick test
T cell – thymus cell
Th cell (T helper cell) – also Th1 and Th2
TNF-α – tumor necrosis factor-α
Treg – regulatory T cell
UK – United Kingdom
U.S. – United States of America
WHCC – Women’s Health Concerns Clinic
1 Introduction

1.1 Outline

Early life experiences, beginning in utero, shape development in ways that profoundly affect health throughout the lifespan. One of the most widely studied exposures in this context is prenatal maternal stress (PNMS). Stress is associated with alterations in cardiovascular, immune, and neuroendocrine systems and when these occur in the context of pregnancy the result can have serious negative health consequences for both mother and child. PNMS is associated with myriad poor obstetric and neonatal outcomes (for review see Graignic-Philippe et al., 2014 or Beydoun & Saftlas, 2008) that are, in turn, associated with disease in adult life (Barker, 2007; Barker, 2006). Furthermore, animal research consistently illustrates that PNMS can exert long-lasting negative ‘programming’ effects on the offspring’s neurodevelopment and stress reactivity (for review see Glover et al., 2010 or Seckl & Meaney, 2004). Recent prospective studies in humans are further supporting the notion that PNMS affects fetal development in ways that greatly influence future development, and hence disease susceptibility, across the lifespan (Sandman et al., 2012; Beijers et al., 2010; Kinsella & Monk, 2009).

Allergic disease represents a major public health concern worldwide, affecting 30-40% of the world population, and prevalence rates are rising (World Allergy Organization, 2011). It is now widely recognized that both genetic and environmental factors contribute to the emergence of allergic disorders. Numerous environmental factors have been linked with the prevalence of allergic diseases, including air pollution, pollen, tobacco smoke, poor diet, and medication use (e.g., paracetamol, antibiotics) (Asher et al., 2010). Among the factors thought to play a role in atopic disease development, psychosocial factors, particularly stress, have garnered increasing interest. This is not surprising, given that allergic diseases were thought to be of psychosomatic origin over a century ago (Dave et al., 2011). In fact, a meta-analysis of the evidence concluded that psychosocial factors are associated with subsequent development of atopic disorder (Chida et al., 2008). Given that some markers
of future allergy development are evident at birth (Warner, 2004; Jones et al., 2000), there has been significant attention in the last 15 years as to whether prenatal maternal stress may confer a greater risk for the development of allergic disease in offspring (Peters et al., 2013; Kozyrskyj et al., 2011; Wright & Enlow, 2008; Wright, 2005; von Hertzen, 2002; Shanks & Lightman, 2001) and whether this may explain, in part, the rising rates of asthma and allergies in developed countries. Accumulating evidence from animal and human studies (see below) suggests that this link exists although the underlying mechanism(s) remain a matter of debate. The most likely candidate is heightened maternal hypothalamic-pituitary-adrenal (HPA) axis function (Beijers et al., 2014; Reynolds et al., 2013; Kozyrskyj et al., 2011; Wright, 2005; von Hertzen, 2002); however, altered maternal immune function has also been implicated (Beijers et al., 2014; Shanks & Lightman, 2001). Nevertheless, prospective studies in humans measuring both psychological and physiologic stress during pregnancy, allergy development in children, and possible underlying mechanisms are needed to shed further light on this issue.

One major flaw with the cross-generational programming research to date, especially studies of PNMS, maternal HPA axis activity and infant health outcomes, is the use of relatively stress-free participants (Beydoun and Saftlas, 2008). It has been suggested that in order to uncover the consequences of PNMS and the mechanisms underlying glucocorticoid (GC) programming, studies should focus on individuals who are exposed to more marked levels of stress (O’Donnell et al., 2009; Bolten et al., 2011) such as those experiencing multiple and/or chronic stressors and traumas (O’Donnell et al., 2009; Glover et al., 2010).

Canadian immigrants are overburdened with stressors such as poverty, social isolation, and trauma relative to their native-born counterparts and therefore represent a population likely to exhibit both poor mental health (George et al., 2015; Hansson et al., 2010; Beiser, 2005) and HPA axis dysregulation in pregnancy. Furthermore since immigrants represent an increasing portion of the Canadian population, there is a need for a better understanding
of the specific factors that contribute to immigrant health (Hansson et al., 2010) including immigration-specific factors such as language barrier. This may aid in understanding the contexts in which immigrants are more vulnerable or more resilient to poor health than non-immigrants.

The current thesis examines whether prenatal maternal stress may predispose infants to allergic disease by prospectively examining pregnant immigrant women and their infants, who represent an at-risk population. Particular attention is paid to psychosocial correlates of prenatal stress in this group, including general and immigrant-specific determinants of health, and potential mechanisms underlying their association with infant atopy. Mechanisms studied here include maternal HPA axis activity, maternal immune parameters, and infant HPA axis activity.

The thesis is organized in a traditional ‘book format’ as the pilot study represents a unified investigation of related objectives (see Section 1.8). Nevertheless, publications arising from the work summarized herein include:


3) Peer M, Soares CN, Levitan RD, Streiner DL, Steiner M. Using a birth cohort to understand complex relationships between mental health and maternal-infant health in pregnant immigrant women: lessons learned from a pilot study. (to be submitted to *J Immigr Minor Health*)
1.2 The Fetal/Developmental Origins of Adult Disease

The research on fetal origins of adult disease arose from the by now well-established ‘Barker Hypothesis’ (Barker, 1992) which stated that type 2 diabetes, stroke, hypertension and coronary heart disease originate as a result of a developmental plasticity in response to under-nutrition in utero. Specifically, maternal nutritional deficiencies during pregnancy were linked to low birth weight, a known risk factor for these adult diseases. Subsequent research with animal models of maternal under- and over-nutrition provided evidence supporting the Barker Hypothesis.

From an evolutionary perspective, the ability of maternal-fetal informational exchange to influence the course of the offspring’s physiological development can be seen as a sophisticated method of improving the offspring’s chance of survival. However, intended influences on one system may lead to unintended changes in other systems and, eventually, to dysfunction and/or disease. Likewise, if the message received by the fetus does not match the postnatal environment, the adaptive response may actually be harmful and contribute to development of disease in adult life (Entringer et al., 2010; Shanks & Lightman, 2001; Coe & Lubach, 2005).

The Barker Hypothesis has given rise to a relatively new field of research known as ‘the developmental origins of health and disease’ (Wadhwa et al., 2009) or the ‘fetal origins hypothesis’ (Barker, 2007). This approach emphasizes the influences of early life environmental exposures on later physiologic structure and function, and provides a powerful theory to guide not only research but also interventions for improved health. The majority of research in this area has focused on the effects of prenatal maternal nutrition and prenatal maternal stress on offspring behaviour, stress reactivity, and susceptibility to neurologic or psychiatric (affective, behavioural) disorders. Relatively less attention, however, has focused on the possible contribution of prenatal stress to immune programming despite the well-known interaction between stress, the hypothalamic-
Newly emerging evidence is highlighting the role of prenatal stress in the development of diverse array of diseases. A recent large-scale study found high PNMS was associated with infant illness (including gastrointestinal, respiratory, and total illness) as well as emergency department visits across the first year of life (Phelan et al., 2015). These results echo those of an earlier, smaller-scale study by Beijers et al. (2010) where prenatal stress and anxiety were associated with greater numbers of infant illnesses as well as with more antibiotic use in the first year of life. Another large population-based cohort study reported that maternal life stress during pregnancy was associated with an increased risk of the first diagnosis of a wide range of offspring diseases including eye, ear, respiratory, digestive, skin, musculoskeletal, and genitourinary diseases (Tegethoff et al., 2011). Given this new evidence, along with a strong theoretical rationale (see section 1.3 below), the possibility that PNMS may program offspring immunity is gaining ground (Marques et al., 2013; Peters et al., 2013; Wright 2007).

1.3 Rationale for Prenatal Stress Programming of Allergy

1.3.1 Interactions between Stress/HPA axis and the Immune System

The hypothalamic-pituitary-adrenal (HPA) axis is activated in response to both physical and psychological stress. This activation involves a cascade of events: the hypothalamus secretes corticotrophin-releasing hormone (CRH), CRH signals the pituitary to release adrenocorticotropic hormone (ACTH), and ACTH acts on the adrenal gland to stimulate the release of glucocorticoids (GCs) into circulation (specifically, cortisol in humans, corticosterone in mice and rats). GCs, in turn, signal back to the hypothalamus and pituitary to inhibit the above-mentioned cascade (a process known as ‘inhibitory feedback’). This negative feedback appears to result from GC binding in specific brain regions, namely the hypothalamus and hippocampus (Smith & Vale, 2006).
From an evolutionary perspective, the purpose of HPA axis activation and subsequent GC release in response to stress is thought to reflect a need to shift physiologic metabolism toward mobilization of energy stores necessary for immediate action (e.g., glucose) and away from those not immediately needed (e.g., reproductive-associated functions). Thus, GCs act on multiple physiologic systems including the digestive, metabolic, reproductive, nervous, and immune system to coordinate a complex whole-body response. With respect to the immune system, the goal of the stress response is to accelerate wound healing and prevent infection by facilitating the elimination of pathogens.

GCs act in several ways to influence gene expression; however, the most well recognized mechanism is through binding to the glucocorticoid receptor (GR). The GR is a nuclear hormone receptor (i.e., ligand-induced transcription factors) that up-regulates gene expression by directly interacting with promoter sequences on DNA, known as glucocorticoid response elements (or GREs) (Liberman et al., 2007; Smith & Vale, 2006). Basal GR expression differs by tissue and immune cell type, reflecting their respective sensitivity to the actions of GCs (Druker et al., 2006) but GRs are expressed extensively throughout the brain (Smith & Vale, 2006). GCs can also control gene expression through protein-protein interactions with other transcription factors including: nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), activator protein-1 (AP1), signal transducer and activator of transcription protein (STAT) and nuclear factor of activated T-cells (NFAT) (Liberman et al., 2007; Druker et al., 2006; Smith & Vale, 2006; Padget & Glaser, 2003).

To help clarify the relationship between the stress and immune systems, a brief overview of the immune system is presented here. The immune system is generally divided into two components, the innate and adaptive responses, each of which includes both cellular and humoral (i.e., antibody-mediated) components. The innate immune component is the non-
specific, fast-acting response that involves phagocytes (macrophages, neutrophils and dendritic cells) and other cells (monocytes, basophils, mast cells, eosinophils), and the molecules that facilitate communication between them (complement, cytokines and acute phase proteins) (Delves & Roitt, 2001a; Parkin & Cohen, 2001). The adaptive (also called ‘acquired’) immune response is a slower but more specific reaction involving B and T cells that ‘remember’ antigens previously encountered (Delves & Roitt, 2001a; Parkin & Cohen, 2001) where an antigen is defined as any molecule recognized by receptors on lymphocytes (Delves & Roitt, 2001a). The distinction between the two arms of the immune system is slightly misleading, however, as there is overlap and interaction between them.

One group of effector T cell, the T helper (Th) cells (distinct from cytotoxic T cells), play a central role in controlling immune responses by secreting cytokines that affect both innate and adaptive immune cells (Kara et al., 2014; Zhu & Paul, 2010). Cytokines are soluble proteins that act as molecular messengers between cells (within the immune system and between the immune and other bodily systems) (Delves & Roitt, 2001a; Parkin & Cohen, 2001) and are extremely important in the orchestration of immune responses. Different types of effector Th cells have been identified based on the profile of cytokines they produce and, consequently, their function (Kara et al., 2014; Jutel & Akdis, 2011; Zhu & Paul, 2010; Parkin & Cohen, 2001). These cells also differ in the cytokine microenvironment that stimulates their differentiation.

Upon leaving the thymus, naïve (i.e., undifferentiated) T cells circulate in the lymphatic system until they encounter an antigen-presenting cell (APC) (Georas et al., 2005). APCs display peptide fragments (i.e., antigenic material) on their cell surface, coupled to a major histocompatibility complex (MHC) molecule, that bind to the surface T cell receptor (TCR) (Georas et al., 2005; Delves & Roitt, 2001a). This binding, in combination with the release of cytokines, chemokines and co-stimulatory signals by activated cells, dictates T cell differentiation (Jutel & Akdis, 2011; Ozdemir et al., 2010; Zhu & Paul, 2010; Georas et al., 2005; Delves & Roitt, 2001a).
Originally two kinds of T-helper cells were identified and classified as pro-inflammatory (Th1 cells) and anti-inflammatory (Th2 cells) (Delves & Roitt, 2001b; Parkin & Cohen, 2001); however, other Th subsets have since been discovered including regulatory T cells (Tregs) and, more recently, Th22 cells and pro-inflammatory Th17 and Th9 cells (Kara et al., 2014; Jutel & Akdis, 2011; Ozdemir et al., 2010). Th1 cytokines are primarily involved in cell-mediated inflammatory responses while Th2 cytokines promote class-switching of B cells to IgE production and clonal expansion of naïve and IgE+ memory B cells (i.e., they promote allergic responses) (Jutel & Akdis, 2011; Ozdemir et al., 2010; Delves & Roitt, 2001b; Parkin & Cohen, 2001). Furthermore, the Th2 cytokines IL-4 and IL-13 lead to production of allergen-specific IgE antibodies by B cells and these antibodies bind to mast cells and basophils, priming them for the characteristic allergic response upon allergen re-exposure (described in greater detail below) (Galli & Tsai, 2012; Ozdemir et al., 2010).

The cytokine environment is critical in determining the fate of a naïve T cell (i.e., which type of Th cell it will become) (Zhu & Paul, 2010; Georas et al., 2005) and since cytokines secreted by one Th subset are self-promoting (and in some cases simultaneously suppressive of another Th subset) (Jutel & Akdis, 2011; Zhu & Paul, 2010), a positive feedback loop occurs that can lead to an imbalance between Th subsets. In fact, the pathogenesis and course of a number of immune disorders (including allergies) have been linked with an imbalance between Th cell subsets (Jutel & Akdis, 2011). Of note, Th1 cytokines suppress Th2 cells and vice versa (Delves & Roitt, 2001b) and allergic disease is characterized by an imbalance between these two subsets, specifically an exaggerated ratio of Th2:Th1 cells and cytokines (Georas et al., 2005).

Briefly, the key events and cellular players involved in allergic reactions are summarized here. An allergic reaction consists of two phases: the immediate hypersensitivity response, happening within minutes of allergen exposure and leading to acute symptoms, and the
late phase which occurs hours after allergen exposure and contributes to chronic inflammation and tissue remodeling (Galli & Tsai, 2012; Jutel & Akdis, 2011). The immediate hypersensitivity reaction occurs when IgE that is bound to mast cells and basophils, via the high-affinity receptor for IgE (FcεRI), cross-links with allergen causing the release of histamine, lipid mediators, chemokines and Th2 cytokines (e.g., IL-4) from these cells (Galli & Tsai, 2012; Jutel & Akdis, 2011; Ozdemir et al., 2010; Georas et al., 2005). The late phase allergic reaction, occurring 6-12 hours later, involves the reactivation and clonal expansion of allergen-specific T cells in response to cytokines and chemokines released primarily by eosinophils (but also by mast cells, basophils, and Th1 cells) (Jutel & Akdis, 2011; Ozdemir et al., 2010). Additionally, migration and infiltration of these cells at the site of allergen exposure during this phase leads to release of mediators which act to further contribute to inflammation (Ozdemir et al., 2010).

There is a well-established bi-directional relationship between stress, the HPA axis and the immune system. In fact, the original observations of Hans Selye that led to discovery of the generalized stress response included atrophy of the thymus (an important immune organ). Thus stress, and especially activation of the HPA axis, is generally considered immunosuppressive and anti-inflammatory (Calgani & Elenkov, 2006; Wilckens & De Rijk, 1997) whereby these suppressive actions of GCs provide a feedback mechanism to control inflammation and other aspects of immunity in order to ultimately restore homeostasis (Liberman et al., 2007; Druker et al., 2006). In fact, these suppressive effects of GCs on inflammation and other aspects of immunity are the mechanism of action of pharmaceutical therapies (e.g., prednisone, betamethasone) for allergic, inflammatory, and autoimmune diseases including asthma, arthritis, colitis, and systemic lupus erythematosus (Liberman et al., 2007; Butts & Sternberg, 2008). In vitro and in vivo studies have shown that these effects are accomplished via a stress- or GC-induced Th2 shift: decreased production of Th1 cytokines and increased production of Th2 cytokines (Montoro et al., 2009; Kemeny & Schedlowski, 2007; Liberman et al., 2007; Calgani & Elenkov, 2006; Segerstrom & Miller, 2004; Padget & Glaser, 2003).
Glucocorticoids affect all aspects of immune cell function including cytokine production, chemo-attractant and adhesion-molecule expression, immune cell differentiation, proliferation, and trafficking, antigen presentation, and immune receptor expression (Kemeny & Schedlowski, 2007; Calgani & Elenkov, 2006; Segerstrom & Miller, 2004; Padget & Glaser, 2003; Wilckens & De Rijk, 1997) (for review see Tait et al., 2008). However, the GC-induced Th2 shift occurs primarily via inhibition of IL-12 (the main inducer of Th1 responses) (Calcagni & Elenkov, 2006; Elenkov & Chrousos, 1999). Activated GC receptors on APCs suppress production of IL-12 (Calcagni & Elenkov, 2006; Druker et al., 2006; Elenkov & Chrousos, 1999) which, in turn, increases production of Th2 cytokines (e.g., IL-4, IL-10, IL-13) and inhibits production of Th1 cytokines (e.g., IL-1, IFN-γ, and TNF-α), although GCs may also directly up-regulate production of Th2 cytokines (Calcagni & Elenkov, 2006). In addition to these effects on cytokine production, GCs have also been shown to affect T cell differentiation in favour of a Th2 shift: inhibiting Th1 cell development and boosting Th2 cell development (Miyaura & Iwata, 2002). Thus GCs lead to an imbalance of Th1/Th2 responses characterized by increased Th2 cytokines and increased Th2 cells (Montoro et al., 2009).

Communication between the immune system and the HPA axis also works in the other direction, with changes in immunity influencing HPA axis activity. Activation of the immune system (e.g., in the acute phase of the inflammatory response) results in increased levels of peripheral pro-inflammatory cytokines (e.g., IL-1, IL-6, TNF-a) which, in turn, stimulate the HPA axis to secret GCs (to keep the inflammatory response in check) (Liberman et al., 2007; Druker et al., 2006; Karrow, 2006).

Of note, the stress-immune interactivity described above is an overly simplified description as it has become increasingly clear that the nature and duration of the stressor are important determinants in the immune outcome. Notably, acute and chronic stress may
result in different (and even opposite) effects on immune function (Dhabhar, 2008; Forsythe et al., 2004; Segerstrom & Miller, 2004; Padget & Glaser, 2003; von Hertzen, 2002). In a meta-analysis of the literature, Segerstrom & Miller (2004) concluded that acute time-limited stressors (e.g., public speaking, mental arithmetic) were associated with upregulation of cellular (Th1) immunity and suppression of specific/adaptive (Th2) immunity, ‘brief naturalistic stressors’ (such as studying for an examination) were associated with a cytokine shift (Th2 shift), while chronic stress was associated with suppression of both cellular and humoral immunity. This is in line with other studies reporting that acute stressors enhance aspects of cell-mediated immunity such as increasing the number of circulating natural killer (NK) cells (Dhabhar, 2008; Schmid-Ott et al., 2001; Breznitz et al., 1998), possibly due to concomitant activation of the sympathetic adrenal-medullary (SAM) in response to acute stress (Tait et al., 2008; Kemeny & Schedlowski, 2007). Although Segerstrom and Miller’s meta-analytic results showed that chronic stress is immunosuppressive, clinically chronic stress and chronic activation of the HPA axis increase inflammatory responses (and consequently aggravate inflammatory/allergic disorders) and are associated with the development of autoimmune disease, both thought to be mediated by the actions of glucocorticoids and/or tissue-specific GR sensitivity (Cohen et al., 2012; Tait et al., 2008; Liberman et al., 2007).

Thus, the effects of HPA axis activation on immunity differ depending on the immune parameter examined (innate vs. adaptive arm, cell counts vs. cell activity, Th1 vs. Th2 cytokines) and the duration and severity of the stressor (acute vs. chronic). Differences are also seen depending on the population studied (healthy individuals vs. individuals with allergic disease). Overall, the communication between the immune system and the HPA axis is extensive and bi-directional, facilitating a physiologic balance that maintains health or an unbalanced state of disease.
1.3.2 Stress, Glucocorticoids, and Allergic Disease

1.3.2.1 Stress Aggravates Allergic Disease

Considering that allergic diseases are characterized by heightened Th2 responses and that stress is also associated with a Th2 shift, it is perhaps not surprising that observational and experimental studies find that psychological distress (including depression and anxiety) is associated with increased symptoms and poor outcomes in allergic diseases (Montoro et al., 2009; Chida et al., 2008). Additionally, within the allergic population negative affect (e.g., anxiety) has been shown to further exacerbate allergic reactions to stressors. For example, Kiecolt-Glaser et al. (2009) found that anxious patients with allergic rhinitis had larger allergic responses after a laboratory stressor compared to less anxious patients. Thus both stress and negative affect appear to worsen allergic reactions and negatively impact the course of allergic disease.

The early years of life are of major importance to the development of allergic disorders (Warner, 2004; Jones et al., 2000), therefore much research has focused on the effects of stress in children with allergies and/or asthma (the most common allergic disease in childhood) and the role of the family environment in childhood atopy (Wright et al., 2005). Overall, psychosocial factors affect atopic disease in children just as they do in adults (Chida et al., 2008). Increased exposure to adverse experiences in the home (e.g., parental divorce, parental death, witnessing domestic violence) has been linked with increased odds of asthma in a recent population-based, cross-sectional study of children aged 0-17 in the U.S. (Wing et al., 2015). Additionally, as in adults, asthmatic children exposed to a severe negative life event experience a significantly increased risk of a subsequent asthma attack. If the acute stressor occurs in the context of chronic stress, the increased risk is particularly high and almost immediate (Sandberg et al., 2000). Likewise, numerous studies link parental stress and/or poor maternal mental health with childhood asthma and/or poor asthma outcomes in children (Pak & Allen, 2012; dos Santos et al., 2012; Barreto do Carmo et al., 2009; Leao et al., 2009; Chida et al., 2008; Shalowitz et al., 2006; Bartlett et al., 2001; Shalowitz et al., 2001; Weil et al., 1999).
Some preliminary evidence suggests that immune alterations may underlie the poor asthma-related outcomes observed following stress. Asthmatic children exposed to chronic life stress show increased Th2-related immune responses (e.g. increased production of IL-5 and IL-13 after *in vitro* stimulation) and increased eosinophil counts (cells that contribute to chronic inflammation) compared to those not exposed (Chen et al., 2006). This parallels findings in adults with asthma where stress was associated with increased airway inflammation in response to antigens (Liu et al., 2002).

The role of social support in childhood atopy has also been explored. Of note, social support appears to be not only an important contributor to mental health in mothers of asthmatic children (Shalowitz et al., 2006), but may also buffer the association between maternal mental health status and childhood wheeze (dos Santos et al., 2012).

Thus in adults and children stress worsens existing allergic disease. In children, acute and chronic stress, parental stress and maternal mental health have been associated with worsening of asthma while social support may have a protective role in these contexts.

### 1.3.2.2 Stress Precedes the Onset of Allergic Disease

In adults, large prospective studies have found that both perceived stress (Rod et al., 2012) and stressful life events (Lietzen et al., 2011; Loerbroks et al., 2009; Kilpelainen et al., 2002) precede the onset of allergic disease. Of interest, certain types of stressful life events are repeatedly associated with subsequent atopic disease while others are not, possibly related to stressor chronicity. Interpersonal conflicts (e.g., divorce or separation, severe conflicts with a supervisor) were linked with asthma onset while the death of someone close (Lietzen et al., 2011; Loerbroks et al., 2009; Kilpelainen et al., 2002), violence (Lietzen et al., 2011) or unemployment were not (Loerbroks et al., 2009). Severe illness in a family...
member was associated with subsequent asthma development in one very large study (Lietzen et al., 2011) but not in an earlier, smaller study (Kilpelainen et al., 2002). The perception of the severity of stress appears to be another important factor in the association between stress and subsequent allergy development. A study in Denmark found a dose-response relationship between perceived stress and subsequent atopic diseases including asthma, allergic rhinitis, and atopic dermatitis in adults with no allergic disease at baseline (Rod et al., 2012). Similarly participants in the top tertile of stress exposure (defined as the sum of participants’ ratings of the severity of each stressful life event experienced) were twice as likely to develop asthma as those not exposed to any life stressors (Lietzen et al., 2011).

The contribution of stress during early childhood to future onset of allergic disease has also been explored. Large-scale studies have found childhood adversities (including maltreatment) are linked with adult-onset asthma even after controlling for multiple salient confounders (Korkeila et al., 2012; Scott et al., 2012; Scott et al., 2008). Of note, the risk appears to increase with a greater number of adversities (Korkeila et al., 2012; Scott et al., 2008). However, even ‘milder’ stressors may be linked with the incidence of future allergic disorders in children. Wright et al. (2002a) found that perceived stress in caregivers, beginning at 2-3 months post-partum, was associated with an increased risk of subsequent wheeze in the infants’ first 14 months of life in a population at risk of developing allergies. A small, earlier study found that parenting difficulties (including postpartum depression and poor marital support) measured when infants were 3 weeks old were significantly associated with asthma at ages 6 to 8 in those predisposed to allergy development (Klinnert et al., 2001). Of interest, at least one small prospective study following families with a history of atopy across the early years of life has found that the course of allergic manifestation may be altered both negatively and positively by the familial environment. Gustaffson et al. (2002) reported that the probability of recovery from atopic illness was 4 times higher in children of families with functional interaction and a good social support network. Similar associations have also been found in populations not at risk for developing allergies. Parental perceived stress has been
associated with an increased risk of subsequent childhood wheeze in a longitudinal cohort study (Milam et al., 2008). Using data from a population-based birth cohort Kozyrskyj et al. (2008) found that continued exposure to maternal depression or anxiety from birth until age 7 was associated with an increased risk of asthma at 7 years of age.

There is some evidence suggesting that the mechanism by which parental perceived stress, and to some extent depression as well, affects atopy development in children is via alterations in the child’s immune system. In infants predisposed to allergies, higher perceived stress in the child’s primary caregiver across the first 2 years of life was associated with a subsequent immune profile characteristic of atopy (i.e., high total IgE levels, and proliferative and cytokine responses to common allergens) (Wright et al., 2004). Likewise in children aged 13 years with and without asthma, parental perceived stress and depression were associated with increased markers of inflammation and in vitro Th2 cytokine production approximately 7 months later (Wolf et al., 2008).

Overall this line of research suggests, as the authors of a recent meta-analysis concluded, that psychosocial factors have “both an etiological and prognostic effect on atopic disorders” (Chida et al., 2008). In particular, there is evidence that during childhood, life stressors and/or parental perceived stress or depression precede the onset of allergic disease. Some preliminary data suggest that these psychosocial factors negatively impact both allergy morbidity and onset via alterations in immunity.

1.3.2.3 Patients with Allergic Disorders Demonstrate Altered HPA Axis Activity and/or GC Resistance

There is substantial evidence that individuals with allergies have aberrant stress responses. Specifically, low levels of cortisol (Bakkeheim et al., 2010; Fei et al., 2004; Fujitaka et al., 2000) and low post-stressor ACTH and/or cortisol (Buske-Kirschbaum et al., 2010; Buske-Kirschbaum, 2009; Buske-Kirschbaum & Hellhammer, 2003; Wamboldt et al.,
2003) have been found in patients (including adults and children) with allergic asthma, rhinitis and atopic dermatitis. Of note, this association between hyporesponsiveness and/or decreased HPA axis activity and allergy is independent of asthma/allergy treatment (e.g., inhaled corticosteroid use) (Priftis et al., 2009). There is even preliminary evidence that the degree of HPA axis hyporeactivity may be linked with the manifestation of allergic disease. A small study comparing patients with allergic rhinitis to non-atopic controls found that not only were cortisol responses to a stressor blunted during pollen season (i.e., when symptoms and inflammation were aggravated) but, in addition, the degree of cortisol attenuation was correlated with symptom severity (Buske-Kirschbaum et al., 2010).

It has been suggested that blunted responsiveness of the HPA axis accounts for the findings that stress exacerbates allergic inflammation and symptoms in atopic disease (described in the preceding section) (Priftis et al., 2009; Buske-Kirschbaum & Hellhammer, 2003). The inability of GCs to act in their characteristic immunomodulatory way (i.e., suppressing inflammation) has been termed ‘glucocorticoid resistance’ (Miller et al., 2002) and is independent of cortisol levels per se. Glucocorticoid resistance can result from exposure to chronic inflammation, chronic GC use (e.g., as part of asthma therapy) (Cohen et al., 2012; Silverman & Sternberg, 2012), and chronic stress (Miller et al., 2002). The primary hypothesized mechanism underlying GC resistance is tissue-specific downregulation of GRs; however, other possible mechanisms include increased binding of cortisol to cortisol binding globulin (CBG), reduced binding of GC to the GR, and reduced GR translocation from the cytoplasm to the nucleus (Silverman & Sternberg, 2012). At least one study did find that GR levels were downregulated in individuals with allergies under stressful conditions. Miller and Chen (2006) found that asthmatic children exposed to acute and chronic stress simultaneously displayed a 5.5-fold reduction in GR mRNA compared to asthmatic children who did not experience such stressors.

In summary, the complex relationship between the HPA axis and the immune system has been studied extensively in the context of allergic disease. Research repeatedly shows that
stress leads to the worsening of atopic symptoms and outcomes in persons with allergies and also temporally precedes the onset of allergic disease. Underlying these observations are changes in immune function that appear to be mediated, at least in part, by the actions of GCs. The importance of the stress-immune relationship in atopy is further supported by research showing that persons with allergies show aberrant HPA axis activity and, in some cases, GC resistance following chronic stress exposure. The reciprocity between the stress and immune systems has extensive implications for not only the course of allergic diseases but also for their development.

1.3.3 Negative Developmental Programming Effects of Prenatal Stress and Glucocorticoid Overexposure

Substantial evidence links prenatal stress with adverse birth outcomes (e.g., preterm or complicated delivery, low birth weight) and with negative effects on short- and long-term physiologic and behavioural development in the infant (Staneva et al., 2015; Buss et al., 2012; Sandman et al., 2012; Fransson et al., 2011; Beijers et al., 2010; Goedhart et al., 2010; Martini et al., 2010; Kinsella & Monk, 2009; Beydoun & Saftlas, 2008; Lobel et al., 2008; Weinstock, 2008; Borders et al., 2007; Talge et al., 2007 Kajantie, 2006; Owen et al., 2005; Van den Bergh et al., 2005; Rieger et al., 2004; Orr et al., 2002). Thus, long-term health outcomes are ‘programmed’ in utero by maternal stresses. The mechanism(s) by which maternal stress programs offspring development and disease susceptibility is not completely understood; however, stress reactivity in the next generation appears to be especially susceptible to the effects of PNMS (Glover et al., 2010), potentially acting as the mediator between PNMS and increased vulnerability to poor health (Seckl & Meaney, 2004).

The maternal stress system undergoes substantial changes during pregnancy. Over the course of pregnancy there is a significant and progressive rise in maternal plasma CRH, ACTH, and cortisol concentrations due to production of CRH by the fetal membranes and placenta. Glucocorticoids play key roles in the differentiation of a wide range of tissues
including the lungs, liver, kidneys, muscle, fat, and gut and are therefore necessary for normal fetal development (Fowden & Forhead, 2004); however, fetal overexposure to GCs can retard fetal growth. To prevent this, the placental enzyme 11-beta-hydroxysteroid-dehydrogenase-2 (11βHSD2) metabolizes cortisol into inert cortisone thereby blocking the majority of maternal cortisol from reaching the fetus (Fowden & Forhead, 2004; Thellin & Heinen, 2003). However, activity and expression of placental 11βHSD2 may vary due to genetic, nutritional, and/or endocrine factors leading to variable rates of transfer of cortisol from mother to fetus (Kajantie & Phillips, 2006; Fowden & Forhead, 2004; Thellin & Heinen, 2003; von Hertzen, 2002). Thus high correlations between maternal and amniotic fluid/fetal cortisol levels may be observed despite the protective barrier provided by placental 11βHSD2 activity (Sarkar et al., 2008; Gitau et al., 2001).

The negative effects of elevated maternal cortisol have been observed following clinical use of exogenous GCs during pregnancy (e.g., in cases of threatened preterm delivery to enhance fetal lung maturation). Exogenous GC administration is associated with poor obstetric outcomes, specifically retarded fetal growth and reduced birth weight (Seckl & Meaney, 2004) independent of preterm labour (Davis et al., 2009).

The implication of these observations is that PNMS may lead to increased maternal cortisol which is then passed on to the fetus, causing impaired growth and development (Reynolds, 2013). Until recently, the majority of studies investigating this hypothesis in humans have used a retrospective design, restricted outcomes to those measured exclusively at birth, and failed to measure physiologic parameters in mother and infant. Newer research, however, has shown high levels of prenatal cortisol directly link prenatal stress/depression not only with negative short-term outcomes (i.e., shorter gestational age, greater risk of preterm birth or prematurity, low birth weight etc...) but also with negative long-term outcomes (i.e., poor infant development, maladaptive infant temperament, altered childhood and adolescent development) (Zijlmans et al., 2015a; Baibazarova et al., 2013; Buss et al., 2012; Bolten et al., 2011; Class et al., 2011; Davis & Sandman, 2010; Davis et al., 2009; Field & Diego, 2008; Van den Bergh et al., 2008; Davis et al., 2007; Sandman et al.,
Accumulating evidence points to altered prenatal maternal HPA axis activity, especially heightened levels of prenatal maternal GCs, as the primary mechanism by which prenatal stress may program offspring development and health (Beijers et al., 2014; Mesquita et al., 2009; Field & Diego, 2008; Van den Bergh et al., 2008; Kajantie, 2006; Owen et al., 2005; Wadhwa, 2005) although this appears to be highly dependent on the type of outcome measured (Zijlmans et al., 2015a).

Thus, PNMS programs health and behaviour in the next generation and maternal HPA axis activity appears to play a key role. This programming is thought to occur by altering the reactivity of important physiologic systems (i.e., neuroendocrine axes) in the offspring (to be discussed in greater detail below, in section 1.5.1). Programming effects may be observed in early life; however, persistent and substantial effects are seen in adulthood as well. Susceptibility to myriad poor health conditions across the lifespan are therefore linked with PNMS and/or maternal HPA axis function during pregnancy.

1.3.4 Allergic Disorders Begin in Early Life

In humans, the majority of immune development occurs in utero and during early postnatal life suggesting this is a critical period for potential programming of allergic disease (Kozyrskyj et al., 2011; Wright 2007; Warner & Warner, 2000). In particular, the antenatal environmental plays a key role in determining atopic outcomes since sensitization to environmental allergens is already present and measurable at birth (Warner, 2004; Jones et al., 2000). It is believed that the fetus is exposed to antigens in utero via amniotic fluid swallowing (thereby affecting immune cells and development of the fetal gut) and/or direct transfer across the placenta (Warner, 2004; Jones et al., 2000).

Individuals destined to become allergic already display altered immune parameters in cord blood including increased Th2:Th1 cytokine-producing cells (Herberth et al., 2010), Th2-
skewed basal cytokine levels (Macaubas et al., 2003), increased proliferative and Th2-skewed responses to stimulants and antigens in vitro (Martino & Prescott, 2011; Chung et al., 2007; Allam et al., 2005; Warner, 2004; Neaville et al., 2003), and altered Treg cell function (Smith et al., 2008). Likewise, markers of future allergy/asthma development have been identified in infancy including positive skin prick test to specific allergens in the first year of life, detectable levels of allergen-specific IgE in early infancy (Van Bever et al., 2008), low levels of IFN-γ (Chung et al., 2007), and elevated TNF-α production in response to LPS (lipopolysaccharide) (Halonen et al., 2013). Thus, exaggerated Th2 immune responses at birth and in the early years of life are markers of future atopic disease development (Ngoc et al., 2005).

In summary, there is a complex bi-directional relationship between stress and the immune system that is driven in part by glucocorticoids. In particular, stress appears to have a significant impact on allergic disorders that includes both worsening of established disease and the promotion of disease development in susceptible individuals. Considering that environmental exposures occurring in early life (including in utero) play a key role in allergy development, prenatal stress (known to be associated with impaired development and disease vulnerability) is likely to contribute to allergic disease development. GCs have numerous important roles during fetal development that greatly impact subsequent health and development. While the effects of stress and GCs are generally immunosuppressive and anti-inflammatory, the context in which they occur (e.g., receptor expression, cytokine microenvironment) are important determinants of the nature and extent of their immunological effects. Thus, glucocorticoids can both inhibit and enhance immune function and since cortisol crosses the placenta it is possible, even likely, that immune development is shaped by this GC with downstream consequences for immunity and immune-related health later in life. Research to date has shown that cortisol mediates at least some of the association between PNMS and adverse health/development in the offspring. Thus, GCs are a key candidate for priming of the immune system during development.
1.4 Evidence for Prenatal Stress and GC Programming of Offspring Allergy

1.4.1 Animal Studies

Animal studies employing various prenatal stress paradigms in several different species have reported effects on offspring immune function (Couret et al., 2009; Gotz & Stefanski, 2007; Vanbesien-Mailliot et al., 2007; Pincus-Knackstedt et al., 2006; Llorente et al., 2002; Tuchscherer et al., 2002; Nogueira et al., 1999; Kay et al., 1998; Sobrian et al., 1997; Klein & Rager, 1995) although results are often conflicting (for a complete review, see Merlot et al., 2008). For example, Gotz and Stefanski (2007) found decreased in vitro stimulated lymphocyte proliferation following PNMS exposure in male rats while Vanbesien-Mailliot et al. (2007) found increased in vitro stimulated lymphocyte proliferation in rats exposed to PNMS. Additionally, some of the immune changes reported are so non-specific that their functional significance is unclear and difficult to interpret. For example, Couret et al. (2009) found that PNMS during late gestation in pigs was associated with decreased numbers of white blood cells, lymphocytes, and granulocytes, and a decreased ratio of CD4+/CD8+ (i.e., T helper to cytotoxic T cell ratio) in piglets. These immune measures represent cell counts including cells of both innate and adaptive immunity and involving both the humoral and cellular arms of the immune system. Lymphocyte counts may reflect gross thymic function (Merlot et al., 2008); however, the downstream consequences for immune development and disease susceptibility are not known.

In a recent review of PNMS programming of offspring immune function in animal studies, Veru et al. (2014) attempted to make sense of these conflicting results. The review outlines a method of between-species comparisons based on chronology of developmental milestones and, using this method, highlights the most significant factors influencing disparate study outcomes – namely timing, type and duration of PNMS, the age and sex of offspring, and the species studied (Veru et al., 2014). An earlier review of the animal
literature in this area also described these same factors as important moderators of PNMS on offspring immune function (Merlot et al., 2008).

Overall the review concluded that there is evidence from animal studies to suggest that PNMS may be associated with decreased Th1 and increased Th2 cytokines (Veru et al., 2014). Of note, two studies using animal models of asthma development have reported increased susceptibility following allergic sensitization in offspring exposed to PNMS (Pincus-Knackstedt et al., 2006; Nogueira et al., 1999). In one study, elevated levels of serum total IgE, and both a systemic and local Th2-skewed immune response were found to underlie the offspring’s allergic disease susceptibility in PNMS-exposed mice (Pincus-Knackstedt et al., 2006). It was concluded that PNMS, via promotion of Th2 responses, may program allergic disease (Veru et al., 2014).

The underlying mechanism(s) by which PNMS may alter immune functioning in offspring remain unclear. As GCs are central to the stress response, animal studies have explored whether altered GC levels and/or function may program immunity in the next generation. Numerous animal studies have shown that exogenous GC exposure in pregnant animals affects immune function in the offspring (Kapoor et al., 2008; Coe & Lubach, 2005; Bakker et al., 1998; Reyes & Coe, 1997, Coe et al. 1996). However, only a few animal studies have employed PNMS paradigms to examine whether changes in HPA axis activity underlie immune programming. One study in rats found lower basal and post-challenge GCs in male offspring (Gotz & Stefanski, 2007) and another study found murine offspring exposed to PNMS had reduced CRH expression in the paraventricular nucleus (PVN) of the hypothalamus, an area of central importance in regulation of the stress response (Pincus-Knackstedt et al., 2006). Alternatively, Couret et al. (2009) found that basal GC levels in piglets were unchanged following PNMS exposure (Couret et al., 2009). Conflicting results likely relate to differences between studies in species examined and study designs, leading an earlier review of the animal studies of PNMS programming of offspring immunity to state that it did not find sufficient evidence that GCs mediate this connection (Merlot et al.,
Nevertheless, the number of studies in this area is too small to draw any firm conclusions.

Programming effects on immune development have also been observed in animal models of prenatal inflammatory stress suggesting changes in maternal immunity may be another key mechanism underlying immune programming by PNMS. In rats, maternal infection and/or inflammation during gestation has been associated with increased levels of maternal and fetal corticosteroids during gestation (Hodyl et al., 2007a) and with subsequently blunted stress and immune responses. Specifically when undergoing an immune challenge offspring exposed to prenatal infection/inflammation demonstrate impaired innate immunity (Hodyl et al., 2011; Hodyl et al., 2008; Hodyl et al., 2007a) and a blunted inflammatory response in both serum and brain samples (Lasala & Zhou, 2007), and a lowered GC response to an immune challenge (Hodyl et al., 2008). In guinea pigs prenatal immune challenge was also associated with attenuated stress responses in the offspring (Hodyl et al., 2007b). Stimulation of the maternal immune system during gestation has also been shown to affect the offspring’s adaptive immunity with one study showing exposed offspring had altered T cell differentiation patterns after in vitro stimulation (Mandal et al., 2011).

Extrapolating results from animal studies to humans must be done cautiously, however, as there are large inter-species differences in the course of physiological development; specifically there are significant differences between species in developmental timelines and landmarks (Veru et al., 2014; Merlot et al., 2008; Shanks & Lightman, 2001). Furthermore, some features of ‘life stressors’ in humans are impossible to induce in animal models (von Hertzen, 2002) and animal studies have shown variable effects on immune parameters depending on stressor type (e.g., neurogenic versus psychogenic stressors) (Veru et al., 2014). Despite these important limitations, the animal literature suggests that prenatal stress is associated with altered immunity in offspring, specifically a Th2 skew in immune responses that may predispose to allergy development. Immune programming
likely results from changes in prenatal maternal immunity but some evidence also suggests altered maternal and offspring stress responses may also play a role.

1.4.2 Human Studies

The number of human studies that have examined the effects of prenatal stress on the programming of offspring’s immunity has grown rapidly over the last five years. As outlined previously, inflammation is particularly responsive to changes in HPA axis activity and may therefore be especially susceptible to the effects of prenatal stress (Shanks & Lightman, 2001). Over a decade ago, it was suggested that prolonged maternal stress might increase the susceptibility to asthma and allergy in genetically predisposed individuals (von Hertzen, 2002; Shanks & Lightman, 2001). von Hertzen (2002) hypothesized that chronically elevated levels of stress hormones during pregnancy might lead to an increased risk of allergic disorders in children through in utero effects on T helper cell phenotype differentiation. Alternatively, others have suggested that abnormal levels of stress hormones during pregnancy may be associated with an increased risk of allergic disease development in the offspring via programming of infant HPA axis activity (Marques et al., 2013). The possible mechanisms underlying PNMS programming of offspring atopic disease development remain an area of active investigation (discussed in section 1.5 below); however, evidence is accumulating that this association does exist in humans (see Appendix A).

To date only two studies, one prospective and one cross-sectional, have examined a link between PNMS and multiple types of atopic disease. A recent analysis of data from the Western Australia Pregnancy Cohort (Raine) study (N = 1,587) found that mothers who experienced adverse events (e.g., separation/divorce, money problems, residential move, death of a loved one) during the second half of their pregnancy had higher odds of asthma and eczema in their children at age 14 (Hartwig et al., 2014). A single adverse event in mid-to late-pregnancy was associated with a doubling of the odds of asthma in their child such that the absolute risk of asthma at age 14 in those exposed to a prenatal adverse event in
mid- or late pregnancy compared to those not exposed increased from 6.7% to 14%. The second study is a large Italian cross-sectional study (N = 3,854) which reported that maternal experience of prenatal stressful life events (specifically mourning, divorce, or job loss) was associated with an increased risk of asthma, eczema, allergic rhinitis, and wheeze in their children aged 3 to 14 (deMarco et al., 2012). The absolute risk in children exposed to a prenatal stressful life event versus unexposed children was significantly greater for asthma (8.9% versus 5.6%), allergic rhinitis (10.9% versus 7.3%), and atopic eczema (29.7% versus 21.1%). Supporting evidence for this finding comes from prospective studies where a single atopic disease is the outcome of interest. In a large prospective cohort (N = 2,203) Sausenthaler et al. (2009) found that PNMS was associated with an increased risk of childhood eczema in the first 2 years of life (absolute risk increased from 16.8% to 22.8%) (Sausenthaler et al., 2009). PNMS was defined as maternal exposure to at least 2 out of 12 possible stressful events, which included some psychosocial/emotional factors but was mostly focused on physical factors. Another prospective birth cohort study (N = 730) found high levels (i.e., the top tertile) of emotional stress in late pregnancy were associated with atopic dermatitis at 2 years of age (absolute risk was 9% compared to a mean of 4.3% in the bottom two tertiles) (Wen et al., 2011). ‘Emotional stress’ was identified via the Short Form Health Survey (SF-36) and consisted of a factor that combined symptoms of depression, anxiety, exhaustion and stress.

The most commonly studied childhood atopic outcomes in the area of PNMS programming, however, are asthma and/or wheeze. The largest such study used population data from Sweden to examine whether maternal bereavement before or during pregnancy was associated with the risk of offspring asthma hospitalization (Khashan et al., 2012). Khashan et al. (2012) accessed data of nearly 3.2 million Swedish citizens giving birth between 1973 and 2004 and found bereavement during pregnancy was associated with an increase of 40% in the relative risk of asthma in the child. In separate analyses confined to children over the age of 6 (when an asthma diagnosis is more reliable) they found the risk was almost 2 fold, and that postnatal bereavement did not have a similar association with asthma risk. Another large-scale investigation using Swedish national registries found
PNMS, in the form of maternal bereavement, was associated with asthma in boys aged 1-4 and 7-12 but not in girls (Fang et al., 2011). The relationship between maternal bereavement and asthma in boys was especially true if the PNMS occurred in the 2\textsuperscript{nd} trimester (Fang et al., 2011).

Two population-based cohort studies provide evidence for a link between PNMS and childhood asthma. Cookson et al. (2009) found maternal anxiety at 32 weeks and, to a lesser extent, also at 18 weeks gestation was associated with asthma in children at 7½ years of age (N = 5,810). While this was true for mothers with the most anxiety compared to those with the least, there was also a trend for a dose response relationship. With increasing quartiles of maternal anxiety at 32 weeks gestation, the rates of asthma in children increased from 9.6\% to 15.7\% (Cookson et al., 2009). These results remained when adjusting for multiple salient confounders and, of particular interest, when controlling for postnatal maternal anxiety. Analyzing data from The Generation R Study (N = 4,848) Guxens et al. (2014) found that psychological distress in mid-pregnancy was associated with wheezing at 1-4 years and with physician-diagnosed asthma at age 6 (although the latter was of borderline significance) (Guxens et al., 2014). This association with wheezing at age 1-4 was true for all three measures of distress (including a global measure of distress, depressive symptoms and anxiety symptoms) and for multiple different wheezing phenotype (including early, late, and persistent wheezing). Two other studies also support a link between PNMS and asthma in the offspring. A small-scale prospective study of women who were pregnant during the Quebec Ice Storm of 1998 (Project Ice Storm) found an increased risk of doctor-diagnosed asthma at age 12 in girls born to mothers who reported greater subjective prenatal stress in response to the disaster (no association was seen with objective stress) (Turcotte-Tremblay et al., 2014). Finally, in an analysis of the relationship between economic changes over time and the development of asthma in children using data from the Western Australia Pregnancy Cohort (Raine) study, Kozyrskyj et al. (2010) found an association between family stress during pregnancy and asthma at age 6 but not at age 14.
Likewise, prospective cohort studies have also found associations between PNMS and childhood wheeze. In a prospective cohort Chiu et al. (2012) (N = 653) reported high levels of stressful life events (SLE’s) during pregnancy were associated with wheeze in the child up to 2 years of age, with a 3- to 4-fold increase in the odds of wheeze in those with the highest level of PNMS exposure versus the lowest exposure. Of note, this study also found an exposure-response relationship between PNMS and increased odds of repeated wheeze in early childhood. The rate of repeated wheeze in children not exposed to prenatal SLE’s was 5.1% and this rate increased to 9.5% in those exposed to 1-2 SLE’s, 17.5% in those exposed to 3-4 SLE’s and 18.3% in children exposed to five or more prenatal SLE’s. Further support comes from a recent prospective cohort of inner-city African American and Dominican women (N = 279) where an association between prenatal psychological distress and childhood wheeze by the age of 5 was identified (Reyes et al., 2011). In this study PNMS was classified as ‘demoralization’ and defined as a composite of anxiety, sadness, poor self-esteem, dread, confused thinking, perceived physical health, hopelessness/helplessness, and psychophysiological symptoms. Particularly relevant, persistent wheeze (a phenotype associated with atopy and physician-diagnosed asthma) was present in 22% of children born to mothers with low prenatal demoralization scores but 28% of children born to mothers with high prenatal demoralization scores (an adjusted OR of 2.69, controlling for multiple maternal and child factors). Finally, a prospective cohort study of urban pregnant women that examined the effects of maternal childhood SES on offspring immune parameters also found PNMS was associated with wheeze in children aged 2 years (Sternthal et al., 2011). Altogether there appears to be strong evidence of a link between PNMS exposure and asthma development in children and/or childhood wheeze.

Although recurrent early wheeze is highly predictive of a subsequent diagnosis of asthma, wheezing is common during the early years of life and may reflect small airways or infections rather than atopy (Wright, 2002b). Thus, some studies of PNMS and childhood
asthma/wheeze have examined atopy as a potential mediator of this association. In the
Cookson et al. study (2009) there was no evidence that allergic sensitization played a role
in the relationship between maternal anxiety and childhood asthma since results were not
different in atopic versus non-atopic asthma. This is in contrast to Hartwig et al. (2014)
where the effect of prenatal adverse events on childhood asthma was particularly strong
for children with atopic asthma (defined by high levels of total IgE in their blood).

Three studies have directly examined whether PNMS is associated with childhood atopy.
Hartwig et al. (2014) found that adverse life events during pregnancy were not associated
with allergen-specific IgE or sensitization (measured by SPT) in children at ages 6 and 14,
and that atopy did not mediate the association between PNMS and atopic disease
development. Likewise, maternal anxiety was not associated with allergic sensitization (by
SPT) in 7-year-old children (Cookson et al., 2009). Finally in a smaller study Reyes et al.
(2011) found no association between maternal psychological distress in pregnancy and
total or allergen-specific IgE in children at ages 2, 3 and 5. These studies suggest that a
non-atopic mechanism may underlie the association between maternal PNMS and
asthma/wheeze in children.

Other studies have examined links between PNMS and atopic outcomes earlier in life,
specifically markers of atopic susceptibility in cord blood (i.e., IgE levels, stimulated Th2
cytokine production) (summarized in Appendix B). Elevated cord blood IgE levels are
associated with an increased risk of subsequent allergic disease development (Wen et al.,
2011; Pesonen et al., 2009). To date, one cross-sectional (Lin et al., 2004) and one
prospective study (Peters et al., 2012) have found that elevated cord blood IgE levels were
associated with prenatal psychosocial and life stress, respectively. One other prospective
study found that cord blood IgE levels were linked with cumulative interpersonal trauma
across the mother’s life but not with life stress confined to the prenatal period
(discrimination, financial strain etc.) (Sternthal et al., 2011). This suggests either chronic
stress is linked with offspring atopy or that a non-IgE-mediated mechanism underlies the link between PNMS and allergic disease susceptibility in offspring.

Mattes et al. (2009) found that cord blood lymphoproliferative responses to allergens were increased in neonates of mothers reporting mild to moderate depression in mid-pregnancy compared to neonates born to mothers with no depression. These neonates also exhibited increased spontaneous cytokine production (IL-6 and IL-10) and increased cytokine production upon stimulation with bacterial and antigen agents. These results were independent of a number of potential confounders and are particularly noteworthy since categorization of maternal mood scores were below a clinical threshold. Thus maternal mood, even at sub-clinical levels, may impact offspring immunity with alterations evident in both basal and stimulated cord blood immune parameters.

Three studies have extending these findings beyond the time of birth: one each through to infancy, adolescence, and adulthood (see Appendix C). O‘Connor et al. (2013a) are the first to report a connection between maternal prenatal anxiety and Th2 skewed in vitro T cell responses to antigens in peripheral blood mononuclear cells from infants aged 6 months. This longitudinal study found that prenatal anxiety predicted decreased IFN-γ responder cell frequencies to hepatitis B vaccine and increased IL-4 responder cell frequencies to tetanus vaccine at 6 months of age (but not at 2 months). O‘Connor also examined humoral responses to hepatitis B and found reduced antibody titers at 6 months of age in infants exposed to prenatal anxiety, controlling for socio-demographic and obstetric factors. Thus, preliminary evidence demonstrates effects of PNMS on humoral and cellular immune responses of cord blood cells and immune cells in early infancy.

Veru et al. (2015) examined lymphocyte numbers and stimulated in vitro cytokine production in adolescents aged 13 years (N = 37) from the Project Ice Storm cohort. They found that objective hardship was negatively associated with total number of lymphocytes
and T helper cells, even when controlling for offspring sex and PNMS timing. Objective hardship was also significantly positively correlated with TNF-\(\alpha\) (\(r = 0.46\)), IL-1\(\beta\) (\(r = 0.42\)), IL-6 (\(r = 0.43\)), IL-4 (\(r = 0.44\)), IL-13 (\(r = 0.49\)) and in regression analyses the associations with TNF-\(\alpha\), IL-4 and IL-13 remained significant. This suggests PNMS may have long-lasting effects on T helper cell numbers and functioning, specifically a skewing towards a Th2 profile.

The only study, to our knowledge, assessing immune outcomes in adults following prenatal stress exposure is a study by Entringer et al. (2008) where healthy subjects provided retrospective reports on whether their mothers experienced any major negative life events while pregnant (e.g., a divorce or a death of someone close). Thirty-four young women (the PNMS group) and 28 age-matched women (the control group) provided blood samples for investigation of stimulated cytokine production. A bias for Th2 immunity was seen in the PNMS group compared to controls, evidenced in the IL-4:IFN-\(\gamma\), even though the numbers and ratios of T cells were not different between groups. The Th2 predominance in women exposed to prenatal stress was hypothesized by the authors to possibly increase their vulnerability to atopic disorders and autoimmune disease later in life.

1.4.3 Explaining Disparate Findings: Differences in study design and mediating, moderating and confounding variables

Overall, there is evidence from cross-sectional, prospective, and population-based studies that various types of PNMS may be linked with the susceptibility to and/or development of atopic diseases in the next generation. The supporting evidence spans a number of different allergic diseases and atopic outcomes and may be observed across the lifespan including at birth, during infancy and in adulthood. However, some studies have provided conflicting results, likely due to differences in study design including the definition of PNMS, the timing of maternal and offspring assessments, and the type of atopic outcome measured. Other methodological differences between studies such as the mediating,
moderating and confounding variables examined may also explain disparate findings across studies. These topics are explored below briefly.

Prior studies examining PNMS programming of atopic disease have conceptualized and measured stress in a number of different ways. Most studies defined stress either objectively (e.g., the experience of stressful life events) or subjectively (e.g., perceived stress), although some studies did distinguish between the two types and measured both. In some cases objective stressors encompassed a wide range of events occurring in daily life while others only examined severely stressful events (e.g., bereavement). Likewise, there is no consensus on the best way to measure subjective stress and it has therefore been measured with a number of different questionnaires across separate studies. Measurement of both objective and subjective stress across a wide spectrum of severity may reveal more clearly whether only certain kinds of stress contribute to immune programming and/or whether a threshold of stressor severity exists.

Another critical consideration when attempting to reconcile disparate findings is whether there are one or more ‘windows of vulnerability’ in pregnancy whereby maternal stress can exert harmful effects on offspring immune development. Indeed the development of different immune cell types occurs at different stages and reviews on the topic of PNMS programming of offspring immunity have concluded that the “sensitive window of immune vulnerability” remains to be determined (Marques et al., 2013; Karrow, 2006). Some studies have identified specific times during gestation when PNMS exposure confers the greatest risk for poor pregnancy outcomes such as a large-scale study by Class et al. (2011) where PNMS that occurred in the 5th or 6th months of pregnancy was implicated. Other studies have reported an association between birth outcomes and the pattern of maternal stress across pregnancy (Glynn et al., 2008). With respect to allergic outcomes, two human studies suggest that mid- and/or late-pregnancy may be particularly sensitive periods during gestation (Chiu et al., 2012; Fang et al., 2011); however, further confirmation from other studies is still needed. Overall this suggests that multiple assessments of maternal
stress throughout pregnancy may be required to properly capture the experience of stress and its physiologic sequelae.

It is also unclear at what age atopic outcomes should be measured in the offspring to best capture the effects of PNMS. On the one hand, markers of future allergy development measured early in life may indicate an effect of PNMS prior to exposure to the myriad other environmental factors known to influence allergic disease development. However, early markers of atopy are not 100% accurate in predicting allergic disease development and allergies can develop at any age. Two studies have found that PNMS is associated with allergic outcomes in children at earlier but not at later ages (Korzyrskyj et al., 2010; Sausenthaler et al., 2009) while another study reported the opposite finding (i.e., a significant association between PNMS and allergic disease development at an older age, 14 years, but not at a younger age, 6 years; Hartwig et al., 2014) highlighting how the age of the offspring may contribute to disparate results between studies measuring outcomes at different ages. Furthermore, it is still unclear whether only certain kinds of allergic disease may be susceptible to PNMS programming or whether atopy (an underlying feature common to all types of allergic disease) is programmable by PNMS. Previous studies have differed on which allergic diseases were examined and at what age. Again this points to a need for more research on PNMS programming of allergy development in the offspring.

Our understanding of how the immune system functions and develops continues to evolve, rendering proper measurement of immune parameters complicated at best. It has been suggested that studies tend to find immune effects only under challenge conditions, not when basal levels are examined (Coe & Lubach, 2005). Thus, whether immune changes reported are those of the system in a resting state or only when it is responding to a challenge must be kept in consideration when comparing study results (Marques et al., 2013). There are also significant differences in results of immune function measured in vivo (which are more difficult to perform and are therefore rarer) versus those measured in vitro. In vitro stimulation of cells may produce different effects than in vivo manipulation.
due to the use of supra-physiological doses and the lack of context in *in vitro* experiments (i.e., lack the presence of physiologic levels of other hormones and factors that interact with the compound/cell of interest) (Wilckens & De Rijk, 1997). Thus results of *in vitro* studies of immune functioning after exposure to PNMS must be interpreted with caution as their clinical significance remains to be elucidated.

An additional contributor to disparate study results is whether moderating variables were examined or controlled for. In particular, maternal atopic status and the sex of the offspring have emerged as important moderators of the association between PNMS and atopic outcomes. Two separate publications analyzing data from the same cohort (ACCESS cohort) found conflicting moderating effects of maternal atopy: Peters et al. (2012) found PNMS was associated with cord blood IgE levels (a predictor of infant atopy) only in atopic mothers while Chiu et al. (2012) found PNMS was associated with child wheeze at age 2 only in non-atopic mothers. Clearly more research is needed to understand the moderating role of maternal atopy in PNMS programming of offspring immunity.

A lack of investigation of sex-specific differences in immune programming by prenatal stress may explain some of the contradictory findings since prenatal maternal stress is known to differentially program the male and female fetus (reviewed in Sandman et al., 2013). Animal studies have shown that adult males may be more likely to demonstrate decreases in cellular immunity in response to prenatal stress while adult females may be more likely to show humoral suppression following PNMS exposure (Llorente et al., 2002; Kay et al., 1998; Sobrian et al., 1997; Coe et al., 1996; Klein & Rager, 1995). This suggests that females may be more likely to display an atopic predisposition following PNMS exposure however at least one study in humans found evidence to the contrary. Fang et al. (2011) found an effect of PNMS (maternal bereavement) on asthma development only in boys, not girls. Sex-specific differences in response to maternal prenatal infection and/or inflammation have also emerged (Hodyl et al. 2011). Future studies on immune
programming by prenatal stress should examine sex-specific effects or at least control for sex of the offspring.

To date, there is a long list of factors associated with atopic disease development including socioeconomic status, exposure to tobacco smoke and traffic-related air pollution, maternal BMI, breastfeeding, race/ethnicity, number of siblings, and pet ownership in early life (Johnson et al., 2002; Kneepkens & Brand, 2010) to name a few. These protective/risk factors may mediate or confound the association between PNMS and atopic disease development in the offspring and thus, whether they were included in statistical analyses is highly likely to play a role in differing results across studies. Studies with sufficiently large sample sizes achieve enough power to control/adjust for many of these factors thereby providing the strongest evidence for an association between PNMS and atopy in the next generation. However, even small studies may also provide some insight, especially if the sample is homogeneous with respect to some of these variables.

In summary, there are many aspects to study design that need to be carefully considered when studying PNMS as a contributor to atopic disease in the offspring. The field as a whole can benefit from more research to better understand the context in which PNMS may increase allergy susceptibility in the next generation.

1.5 Mechanisms of Prenatal Stress Programming of Offspring Allergy

1.5.1 Alterations in Maternal and/or Infant HPA axis

Animal studies illustrate that PNMS can exert long-lasting programming effects on offspring neurodevelopment and stress reactivity, and the maternal HPA axis appears to play a crucial role in this programming (for review, see Glover et al., 2010). Likewise in humans, cortisol has been called “the culprit prenatal stress variable” (Field & Diego, 2008)
with numerous studies reporting that PNMS (including stress, anxiety, and depression) is associated with altered maternal HPA axis activity and/or cortisol levels (Voegtline et al., 2013; O’Keane et al., 2011; Davis & Sandman, 2010; Pluess et al., 2010; Suglia et al., 2010; Sarkar et al., 2006; Obel et al., 2005; Lundy et al., 1999). The central role of maternal cortisol in various child outcomes has been disputed recently, however, since many studies have failed to find a link between maternal psychological distress and maternal cortisol levels/activity during pregnancy (Zijlmans et al., 2015b; Baibazarova et al., 2013; Hompes et al., 2013; Spicer et al., 2013; Pluess et al., 2012; Bolten et al., 2011; Goedhart et al., 2010; D’Anna et al., 2009; Harville et al., 2009; Kivlighan et al., 2008; Shea et al., 2007; Ruiz et al., 2001 – for review see Zijlmans et al., 2015a). This remains an active area of research considering the wide variation between studies in methodologies and these conflicting results.

With respect to the programming of offspring immune development, to date only a few studies have examined prenatal maternal cortisol in relation to atopic outcomes in the offspring. Two studies found that blunted prenatal cortisol activity is associated with asthma-related outcomes in infants. In a prospective study of healthy women with full-term deliveries, Beijers et al. (2010) reported that more pregnancy-specific hassles and a smaller cortisol decline from waking to bedtime in late pregnancy (37 weeks gestation) were each associated with more infant respiratory illnesses in the first year of life. Wright et al. (2013) observed that increased night-time cortisol in the 3rd trimester was associated with repeated wheeze in children at 2 years of age. Further analyses revealed a flatter afternoon cortisol slope was associated with wheeze in the child in obese mothers only. One other study found that high prenatal maternal cortisol at mid-day plus high cumulative stress in late pregnancy (37 weeks gestation) was associated with more allergic reactions in the first 4 months of life, although small sample size resulted in this being not statistically significant (Zijlmans et al., 2015b). One study provided a conflicting result: O’Connor et al. (2013a) found that prenatal anxiety was linked with decreased adaptive immunity in infants aged 6 months but that prenatal maternal diurnal cortisol did not underlie these results. This disparate finding may be related to the use of a different
measure of prenatal cortisol (diurnal AUCg) which represents a distinctive aspect of HPA axis functioning (i.e., total hormonal secretion) than the previous studies where an effect on infant immunity was shown (i.e., diurnal slope, measuring the pattern of cortisol across the day, and singular cortisol values).

Thus preliminary evidence suggests that maternal prenatal cortisol may play a role in PNMS programming of the infant’s immune development although the underlying mechanism(s) remain unclear. Maternal cortisol may be transferred to the fetus and act directly on fetal immune structures (e.g., the thymus) or cell lineages (e.g., T cells). Alternatively, prenatal cortisol may affect placental function (e.g., placental CRH production) which then negatively affects birth outcomes (e.g., preterm birth, very low birth weight) (Wadhwa et al., 2011), thereby imparting a vulnerability to allergic disease during childhood (Sonnenschein-van der Voort et al., 2014; Chandran et al., 2013). Finally, maternal cortisol may program activity of the infant’s HPA axis which then confers a greater risk of allergy development. It has been suggested that HPA axis programming is ‘strongly implicated’ in the programming of offspring immunity and susceptibility to future disease (Karrow, 2006) with changes in infant HPA axis functioning having been called “the most popular candidate mechanism” for PNMS programming of immune function (Marques et al., 2013). It should be noted, however, that PNMS programming of the infant’s HPA axis can also occur independent of prenatal maternal cortisol.

Human studies have linked PNMS with altered HPA axis activity in the next generation spanning infancy, childhood, and adulthood (Ping et al., 2015; O’Donnell et al., 2013; Davis et al., 2011; Tollenaar et al., 2011; de Bruijn et al., 2009; Entringer et al., 2009; Gutteling et al., 2005; Gutteling et al., 2004; Huot et al., 2004). In general, prenatal stress (especially in mid- to late-pregnancy) and prenatal depression are associated with increased basal plasma corticosterone levels and/or increased HPA responsivity in the next generation (Zijlmans et al., 2015a) although blunted HPA axis activity has also been reported (O’Connor et al., 2013b). The effects of PNMS on HPA axis functioning in offspring depend
on the stage of pregnancy during stress exposure, the nature of the prenatal stressor, the sex of the offspring, and the stress parameters examined in the offspring (Karrow, 2006). HPA axis programming may result from: i) changes in maternal physiology (e.g., altered maternal GC levels), ii) organizational/structural changes to key neuroendocrine organs in the offspring, iii) functional changes in fetal/infant stress reactivity (i.e., a modified set-point and/or distorted central negative feedback sensitivity) and/or iv) disturbances of placental function (Beijers et al., 2014; Marques et al., 2013; Reynolds, 2013; Glover et al., 2010). Evidence for each of these will be (briefly) examined in turn.

Maternal GCs are implicated in HPA axis programming by studies of exogenous GC treatment. In a variety of animal models, exogenous prenatal GC treatment led to changes in HPA axis function that persist into adulthood (Waffarn & Davis, 2012). In humans, exogenous use of GCs in pregnancy also resulted in aberrant offspring HPA axis function in a dose-dependent manner (Waffarn & Davis, 2012). Finally, elevated physiological levels of GCs in animal and human studies have also been linked with altered stress reactivity in the next generation (O'Connor et al., 2013b; Seckl & Meaney, 2004).

Structural changes in the child’s brain have been linked with prenatal distress and/or prenatal cortisol levels. For example, prenatal depression and elevated prenatal cortisol in early pregnancy have been associated with alterations in right amygdala volume and microstructure in neonates and children (Rifkin-Graboi et al., 2013; Buss et al., 2012). This may impact stress reactivity in the child given that the amygdala plays an important role in modulating various physiologic aspects of the stress response (Ulrich-Lai & Herman, 2009; Smith & Vale, 2006). In primates, reduced hippocampal volume has also been reported in offspring following prenatal stress (Coe et al., 2003). Finally, higher prenatal depressive symptoms were recently found to be associated with reduced corticol thickness in right inferior frontal and middle temporal regions in exposed children, possibly representing premature brain development (Lebel et al., 2016).
Epigenetic regulation of the GC receptor may also underlie functional changes in the offspring’s HPA axis activity and preliminary evidence suggests this can be influenced by in utero exposure to maternal stress. Animal models have shown that PNMS is associated with lowered GC receptors in brain regions that regulate feedback of HPA axis activity such as the hippocampus (Weinstock, 2005). Oberlander et al. (2008) provided the first evidence in humans which showed that maternal depression in late pregnancy was associated with methylation of GCR in cord blood, and that this methylation was in turn associated with cortisol stress responses in infants at the age of 3 months. Similarly, Hompes et al. (2013) reported that prenatal anxiety and depression in early- and mid-pregnancy, and maternal cortisol levels in mid-pregnancy were each associated with the methylation of the promoter region of the GC receptor in cord blood.

Finally, PNMS may confer changes on the infant’s HPA axis through changes in placental 11βHSD2 expression and/or activity, independent of maternal levels of GCs. Glover et al. (2009) found that at 17 weeks gestation, maternal and amniotic fluid cortisol levels were highly correlated in the most anxious mothers. On the other hand, Baibazarova et al. (2013) found that while maternal cortisol levels were related to amniotic fluid cortisol levels, neither were associated with maternal stress and/or anxiety. Finally, O’Donnell et al. (2012) found that maternal anxiety at the end of pregnancy was negatively associated with placental 11βHSD2 mRNA expression. Women with the highest levels of anxiety exhibited 30% lower placental 11βHSD2 expression than those with the lowest levels of anxiety. While the first two studies only indirectly examined the association, the latter is the first study in humans to demonstrate a direct link between maternal psychological state and fetal exposure to maternal cortisol via down-regulation of this key placental barrier enzyme.
Central to the idea that HPA axis programming underlies the connection between PNMS and atopic outcomes in offspring is whether HPA axis dysregulation precedes allergic disease onset. Both newborns and infants with an atopic predisposition (i.e., maternal and/or paternal history of allergic disorder) were found to have an increased cortisol response to stress (Ball et al., 2006; Buske-Kirschbaum et al., 2004). Likewise, results of a birth cohort study demonstrated high levels of cortisol were associated with the risk of IgE sensitization to allergens, eczema, and (to a lesser extent) food allergy in the first two years of life (Stenius et al., 2011). Further analyses to investigate the specific temporal pattern found a trend for an association between higher afternoon salivary cortisol at 6 months of age and subsequent sensitization at 12-24 months (Stenius et al., 2011). The authors suggest that perhaps hyperreactivity of the HPA axis activity seen in those predisposed to atopy switches to hyporeactivity before or during the onset of the allergic disease.

1.5.2 Alterations in Maternal Immunity

Another possible mechanism through which maternal stress might program offspring neurodevelopment and/or immunity is via altered maternal immunity (Beijers et al., 2014; Veru et al., 2014; Hodyl et al., 2011; Merlot et al., 2008). Pregnancy is a unique immunologic phenomenon inasmuch as the maternal immune system does not reject a ‘foreign’ object, the fetus. This requires complex coordination of both maternal and fetal factors including a predominance of uterine NK cells and altered levels of complement regulatory proteins in the decidua, and altered expression of the major histocompatibility complex (MHC) on fetal cells (Poole & Claman, 2004). Furthermore, the immunologic milieu changes across gestation. For example, decreased Th1 and increased Th2 cytokines at the maternal-fetal interface in the early stages are necessary for the establishment of pregnancy (Holtan et al., 2015; Poole & Claman, 2004); however, in the maternal periphery a shift towards an inflammatory profile has been observed as pregnancy progresses, (Holtan et al., 2015) culminating in labour which is considered an inflammatory process (Gomez-Lopez et al., 2014).
Perturbations of maternal immunity, evidenced by high levels of pro-inflammatory cytokines and other mediators, are associated with spontaneous abortion, preterm birth (PTB), gestational hypertension and preeclampsia (Christian, 2012; Hodyl et al., 2011; Aagaard-Tillery et al., 2006). Additionally it has been suggested that inflammatory stress during key developmental periods, both in utero and in early life, may permanently program the fetal neuroendocrine-immune axis such that the offspring is predisposed to allergy development (Marques et al., 2013; Karrow, 2006; Shanks & Lightman, 2001). Findings from a recent study support this idea; Illi et al. (2014) found that even relatively mild maternal infections during pregnancy (repeated common colds) were associated with an increased risk of asthma in children at the age of 5.

As outlined in section 1.4.1, animal models have demonstrated that stimulation of maternal immunity during gestation is associated with altered levels of maternal GCs and cytokines with downstream effects on the offspring’s immune responses and immune development. Animal models have also shown that maternal immune stimulation is linked with numerous changes within the placenta including levels of maternally-derived cytokines, immune cell numbers and endocrine factors (Hsiao & Patterson, 2011). Furthermore, there is preliminary evidence in mice that mild inflammation enhances the migration of maternal immune cells across the placenta, a phenomenon termed maternal microchimerism (Wienecke et al., 2012). Cells of maternal origin have been shown to persist into adulthood (Loubiere et al., 2006) and while a clear understanding of the function of maternal microchimerism is still lacking, it has been implicated in the establishment of immune tolerance to allergens (Mold et al., 2008). There is considerable bi-directional communication between the mother and the developing fetus and this includes a substantial exchange of immune-related information (reviewed in Arck & Hecher, 2013); however, the relevance to allergic disease development remains to be elucidated.
Cytokine levels and/or the balance of Th1 to Th2 cytokines in maternal circulation may contribute to fetal immune programming since cytokines can both cross the placenta and alter placental function (Marques et al., 2013; Aagaard-Tillery et al., 2006). A prospective study of modest size (N=90) by Kim et al. (2008) found that the Th1:Th2 cytokine ratio in maternal circulation at mid-gestation was associated with atopy and wheeze in children at the age of 3. Specifically, a Th2 skew in mothers of children with atopy (low IFN-γ/IL-4 ratio) and in those with wheeze (low levels of TNF-α and IFN-γ) but no association with childhood asthma was observed. Another longitudinal study of nearly twice the sample size (n = 178) found high levels of IL-5 and IL-13, both Th2 cytokines, in maternal serum at the end of pregnancy were associated with asthma-like symptoms in infants aged 6 and 12 months (Soto-Ramirez et al., 2012).

Other studies have found a link between atopic outcomes in infants and maternal prenatal cytokine responses, rather than basal levels per se. In a larger birth cohort study (N=353) Herberth et al. (2011) examined stimulated cytokine production in maternal blood during the 3rd trimester, in cord blood, and in blood from the child at 1 year of age. They observed associations between multiple prenatal maternal inflammatory measures and those same measures in children at 1 year of age including IL-10, TNF-α, and IFN-γ/IL-10. Additionally, prenatal maternal stimulated TNF-α was negatively associated with sensitization to inhalant allergen (i.e., specific IgE levels) independent of maternal prenatal IgE. Another large birth cohort study (N=346) found stimulated maternal cytokine production (IL-13, IL-17E, and IFN-γ) was associated with reduced Treg numbers in cord blood which was, in turn, associated with a higher risk of atopic dermatitis and sensitization against food allergens at 1 year of age (Hinz et al., 2012). Thus, preliminary evidence suggests that both basal and stimulated prenatal cytokine levels in maternal blood are associated with the immune profile and allergic susceptibility of the child.

Thus, any link between maternal mood/stress and markers of maternal inflammation during pregnancy suggests a mechanism whereby PNMS may program the offspring’s
propensity for allergic disease. To date, however, studies that have examined this possibility have found mixed results. Coussons-Read et al. (2007) found high levels of stress in early pregnancy were associated with high serum levels of IL-6 and lower IL-10. A later study by the same group found both overall and pregnancy-specific stress were associated with increased TNF-α, but not IL-6, in early and late pregnancy (Coussons-Read et al., 2012). In contrast, Latendresse et al. (2013) found a negative correlation between perceived stress and IL-1 β, IL-6, IL-10, and TNF-α at 14-20 weeks. Thus, prenatal stress has been linked with elevated and lowered levels of inflammatory cytokines (IL-6 and TNF-α); however, two separate studies have found an association with low levels of IL-10 (an anti-inflammatory cytokine). Other studies have found no relationship between prenatal maternal stress and serum cytokines in early- to mid-pregnancy (Christian et al., 2009), mid-pregnancy (Shelton et al., 2015), and late pregnancy (Cheng & Pickler, 2014). Conflicting results are likely due to differences between studies in sample characteristics, stress measures, and the timing of cytokine measurement.

Studies investigating maternal cytokine levels in relation to prenatal depression and/or anxiety have also been inconclusive. Prenatal depressive symptoms were associated with higher maternal serum levels of IL-6 and TNF-α in two studies (Azar & Mercer, 2013; Christian et al., 2009) and higher IL-1β (but not with TNF-α levels) in another study (Cassidy-Bushrow et al., 2012). To confuse matters further, Latendresse et al. (2013) found depressive symptoms and pregnancy-specific anxiety were associated with low levels of IL-1β, IL-6 and IL-10. Finally, other studies have found no association between prenatal depressive symptoms and serum cytokines (Blackmore et al., 2014; Cheng & Pickler, 2014; Okun et al., 2013; Blackmore et al., 2011).

Of interest, however, the study by Coussons-Read et al. (2007) reported that high PNMS predicted higher in vitro IL-1β and IL-6 production by stimulated lymphocytes in late pregnancy suggesting PNMS may be associated with a greater propensity for an
inflammatory response in immune cells exposed to environmental stimuli regardless of circulating basal cytokine levels. Further research on the association between prenatal cytokine levels and prenatal maternal stress is needed, especially since preliminary evidence suggests cytokines may mediate the relationship between PNMS and poor birth outcomes (e.g., GA at birth) (Coussons-Read et al., 2012).

Alternatively, changes to other maternal immune parameters during pregnancy may contribute to allergy in the offspring. Although it was originally believed that IgE does not cross the placenta, placental IgE has been observed and although its function remains unclear (Rindsjo et al., 2010), it is correlated with maternal IgE and appears to be of maternal origin (Joerink et al., 2009). IgE has also been found in amniotic fluid in proportion to maternal IgE and resulting in fetal exposure via amniotic swallowing, aspiration into the respiratory tract and fetal skin permeability (Warner & Warner, 2000). It is therefore not surprising that prenatal maternal IgE levels are correlated with levels of IgE in cord blood (Herberth et al., 2011; Sternthal et al., 2009; Liu et al., 2003) although no correlation has also been reported (Joerink et al., 2009). Nevertheless, maternal prenatal IgE levels are associated with IgE levels and atopy in the infant (Herberth et al., 2011; Liu et al., 2003). It has therefore been suggested that any factors leading to altered maternal IgE levels could impact the risk of atopy in infants (Wright, 2007). To date, no studies have examined whether maternal stress and/or mood factors are associated with prenatal maternal IgE levels.

1.5.3 Other Possible Mechanisms

In the preceding sections, the mechanism by which PNMS may program immunity primarily focused on functional changes in maternal and offspring neuroendocrine and immune systems. The precise way these alterations come about remains to be elucidated but epigenetic mechanisms are implicated. Differentiation of various T cell subsets has been shown to be influenced by epigenetic modifications (Prescott, 2010) and, as outlined previously, Th2-skewed T cells and responses are characteristic of allergic disease.
Epigenetic modification of gene expression is thought to underlie the programming effects of maternal environmental exposures on the offspring’s immune development (Jenmalm, 2011), and this includes how PNMS is proposed to program offspring immunity (Wright 2012). Two recent studies, by the same group of authors, have provided the first human evidence correlating PNMS with long-lasting epigenetic signatures in the offspring. Both studies investigated the epigenetic profiles of adolescents (mean age = 13 years) born to participants from the Project Ice Storm. In the first study, objective hardship (but not subjective distress) was correlated with DNA methylation patterns in multiple types of immune cells including T cells (N = 36) (Cao-Lei et al., 2014). In the second study, DNA methylation signatures in T cells of these same participants (N = 34) were examined in relation to their mother’s cognitive appraisal of the ice storm\(^1\). Methylation profiles differed between adolescents whose mothers’ appraisals were negative versus neutral/positive and a majority of the genes implicated are heavily involved in immune function (Cao-Lei et al., 2015). There was significant overlap with the genes identified in the first study however novel genes and pathways were found to be methylated in relation to cognitive appraisal. Thus, epigenetic changes were evident in immune cells of offspring exposed to PNMS and these changes persisted into adolescence and differed depending on the specific type of stress.

Another highly plausible mechanism underlying PNMS programming of offspring immunity that deserves future research attention involves changes to the maternal or fetal autonomic nervous system (ANS) (Wright, 2012; Karrow, 2006). Both the sympathetic and parasympathetic branches of the ANS contribute to immune regulation (Kenney & Ganta, 2014) but the sympathetic-adrenal-medullary (SAM) axis is particularly implicated in PNMS programming inasmuch as it is primarily activated in response to stress (along with the HPA axis). Furthermore, lymphoid organs are primarily innervated by sympathetic,

\(^1\) Specifically, participants rated the consequences of the ice storm for them and their families on a 5-point scale where responses ranged from ‘very negative’ to ‘very positive’. This appraisal was given during pregnancy, close to the time of the natural disaster.
rather than parasympathetic, nerves (Elenkov et al., 2000). Catecholamines (the end points of the SAM) drive a Th2 shift by inhibiting production of pro-inflammatory cytokines by APCs and Th1 cells while increasing production of IL-10 production and TGF-β through stimulation of β2-adrenergic receptors (β2ARs) (Elenkov, 2007; Calcagni & Elenkov, 2006; Elenkov & Chrousos, 1999). Thus, β2-ARs, expressed on Th1 cells but not on Th2 cells, potentiate the activity of GCs on immune function (Calcagni & Elenkov, 2006; Elenkov & Chrousos, 1999). Cytokines, particularly pro-inflammatory IL-1β and TNF-α, also affect sympathetic activity (Kenney & Ganta, 2014). There is preliminary evidence that individual differences in immune responses to psychological stress are associated with sympathetic reactivity (Steptoe et al., 2001). This suggests that the responses of the SAM and immune systems to stress may be linked, indicating that stress during critical periods of development may program SAM and/or immune functioning and subsequent vulnerability to disease. Catecholamines may play a role in PNMS programming of allergy development but this has not been investigated extensively to date (Veru et al., 2014; Merlot et al., 2008).

Additionally, several other factors are released in response to stressful conditions including prolactin, growth hormone, and nerve growth factor (Webster et al., 2008). Given that receptors for these hormones are present on immune cells (Ignacak et al., 2012; Lambiase et al., 2004; Meazza et al., 2004), downstream effects on immune function are possible. Two other hormonal mediators have been proposed for PNMS programming of offspring immunity, specifically placental CRH and progesterone.

Placental secretion of CRH into both maternal and fetal compartments results in an exponential increase in CRH in maternal circulation in the 2nd half of pregnancy (Majzoub, 2006; Mastorakos & Ilias, 2003) that contributes to maternal prenatal hypercortisolism (Kalantaridou et al., 2010). Placental CRH participates in a positive feedback loop involving the fetal HPA axis whereby placental CRH leads to increased fetal ACTH production which, in turn, increases fetal cortisol output that then further stimulates placental CRH production (Majzoub, 2006). Since placental CRH secretion is stimulated by
glucocorticoids and cytokines (Kalantaridou et al., 2010), prenatal stress may program offspring immune and/or HPA axis function via actions of these substances on placental CRH production. Indeed, levels of salivary cortisol and CRH in maternal blood are significantly correlated during pregnancy and depressed women have been observed to have significantly elevated CRH levels (O'Keane et al., 2011).

Progesterone is well-known to be a strong modulator of the immune system (von Hertzen, 2002; Hellings et al., 2003), and this immunomodulation is thought to play a role in the establishment and/or maintenance of pregnancy (Poole & Claman, 2004). At high levels, such as those occurring during pregnancy, progesterone can shift Th1/Th2 cell differentiation towards Th2 dominance (Miyaura & Iwata, 2002) and alter cytokine production by T cells toward a Th2 bias (Lissauer et al., 2015). As the placenta is a major source of progesterone during pregnancy, one can speculate that alterations in placental progesterone production may have immunomodulatory effects on fetal development. Decreased maternal progesterone following prenatal stress has been proposed to contribute to fetal immune function such that a propensity for allergic disease develops in the offspring (Slano et al., 2011) because lower maternal progesterone during pregnancy was associated with an increased risk of atopic dermatitis in girls aged 0-3 years (Pincus et al. 2010). The mechanism underlying this association is not known; however, it has been suggested that actions of progesterone on prenatal maternal cytokine production may impact immune development in the offspring (Merlot et al., 2008).

Other placental functions may be altered by maternal stress and/or GCs. Indeed, maternal stresses (e.g., hypoxia, caloric excess) have long been known to affect placental gene expression in ways that profoundly shape fetal development (Gheorghe et al., 2010). For example, Xu et al. (2005) found decreased Th1:Th2 cytokine production by placental tissues cultured with exogenous GCs.
Another intriguing possibility for how stress may program offspring immunity comes from two prospective studies showing that stress may exacerbate the negative effects of harmful environmental exposures. A prospective study of children aged 5-9 years found that parental stress influenced the likelihood of asthma development following exposure to traffic-related environmental pollution or in utero tobacco smoke (Shankardass et al., 2009). In another study PNMS showed an interactive effect with environmental allergen exposures that differed depending on maternal atopic status. Specifically, in atopic mothers exposed to high dust mite levels PNMS was associated with increased cord blood IgE levels. In non-atopic mothers, however, PNMS was associated with increased cord blood IgE only in those with low dust mite exposure (Peters et al., 2012). This requires further study, but suggests complex gene by environment interactions contribute to allergic disease development.

Another possible mechanism for PNMS programming of allergic disease susceptibility is via susceptibility to affective disorders. Korkeila et al. (2012) found that the relationship between childhood adversities and asthma in adulthood was significantly attenuated when controlling for psychiatric morbidity. Likewise, a large-scale cross-sectional study by Scott et al. (2008) found that early-onset of childhood depressive/anxiety disorders was predictive of adult-onset asthma. Prenatal stress may result in structural and/or functional changes in the offspring CNS associated with a vulnerability to psychiatric disorders which, in turn, predispose to allergic disease development.

Finally, changes in the infant gut microbiome have also been implicated in the programming of offspring immunity (Zijlmans et al., 2015b; Wright 2007; Karrow, 2006; Warner & Warner, 2000) and PNMS was recently shown to contribute to the development of atopic diseases via this mechanism. Zijlmans et al. (2015b) showed that PNMS was associated with a pattern of infant gut microbiota suggestive of inflammation (i.e., more pathogenic strains and fewer lactic acid bacteria and Bifidobacteria) which was, in turn, associated with more reported allergic reactions through to 16 weeks of age (Zijlmans et
al., 2015b). Of particular interest, these results occurred following subjective PNMS or elevated maternal cortisol levels and there was a relative gradient in the effect of PNMS/cortisol on the infant’s intestinal microbiota (Zijlmans et al., 2015b). A recent study in rats supports this finding and additionally showed that prenatal stress-induced changes to gut microbiota and alterations in multiple physiologic systems (including hyper-reactivity of the HPA axis) extend into adulthood. Furthermore, Golubeva et al. (2015) found that in offspring exposed to PNMS the abundance of certain bacterial strains (e.g., lower levels of lactic acid bacteria) was correlated with HPA axis reactivity in adulthood.

1.6 Modulators of PNMS Programming of Atopic Outcomes

Postnatal environmental exposures have also been shown to affect the development of the immune system and, consequently, the development of allergic diseases. Some of the most well-studied postnatal environmental factors include maternal and infant nutrition (including breast-feeding) (Marques et al., 2013; Prescott, 2010; Karrow, 2006), maternal care/postnatal stress, indoor and outdoor air pollution (Peters et al., 2013), and early-life infection (Kozyrskyj et al., 2011). Nutritional status of the mother is of central importance in the programming of multiple physiologic systems, including the immune system (for review see Marques et al., 2015). PNMS-associated changes to the maternal diet during pregnancy or to placental function could impact the fetus’s nutritional status and, consequently, immune development. Maternal diet may also impact offspring immunity indirectly via HPA axis programming since, for example, high protein intake during late pregnancy has been associated with increased cortisol responses to psychological stress in offspring (Reynolds et al., 2007). The influence of maternal nutrition extends to the postpartum period in dyads who breastfeed as breast milk has antimicrobial properties and many other factors (e.g., long-chain polyunsaturated fatty acids, cytokines) that contribute to the development and maturation of the infant immune system, including effects on macrophages, neutrophils, lymphocytes, and cytokines (Agarwal et al., 2011; Field, 2005). Furthermore, in monkeys, correlations between levels of cortisol in saliva and breast milk have been observed (Sullivan et al., 2011) suggesting a mechanism by which
postpartum maternal distress may influence the development of infant immune and/or stress systems.

The quality of maternal care is another important consideration in the developmental programming literature since maternal care mediates the effects of environmental conditions on offspring physiological development and disease susceptibility (Fish et al., 2004; Teunis et al., 2002). Animal studies have demonstrated that gestational stress can compromise maternal care post-natally, potentially stressing offspring and thereby programming immunity (Merlot et al., 2008). In humans, as described in section 1.3.2.2, postnatal stress has been associated with allergy development/morbidity, and prenatal maternal psychological distress is a well-known risk factor for postnatal maternal distress (Milgrom et al., 2008; Robertson et al., 2004). Thus, prenatal and postnatal environmental exposures interact with one another to influence physiological outcomes (Shanks & Lightman, 2001), adding an additional layer of complexity to the study of PNMS programming of offspring immunity.

Specifically concerning the relationship between stress and allergy development, stressor severity appears to be an important modulator. High levels of stress appear to show stronger associations with allergic disease development (described briefly in section 1.3.2.2). One criticism of the PNMS and programming research to date (especially studies investigating whether maternal HPA axis activity is the underlying mechanism) is the inclusion of relatively stress-free participants - that is, healthy well-educated, middle- or upper-class Caucasians (Beydoun and Saftlas, 2008). Some authors have suggested that focusing on marked levels of stress may yield more definitive conclusions on the role of maternal cortisol in linking PNMS/prenatal depression with poor health outcomes in children (O’Donnell et al., 2009; Bolten et al., 2011). This has led some research groups to centre their studies on more ‘vulnerable’ populations such as women of low socioeconomic status and/or women who are members of a visible minority group.
For example, several of the longitudinal studies on PNMS programming of offspring immunity (from 2 separate research groups) have been based on a prospective cohort sample of urban, low SES, minority women (see Tables 1, 2 & 3). An additional element worth considering when selecting a sample for research on immune programming is that of trauma exposure. As Wright & Enlow (2008) wrote:

...trauma may be a particularly robust potentiator of the cascade of biological events that increase vulnerability to atopy and may help explain the increased risk found in low-income urban populations. (page 536)

To date, only one study of PNMS programming of allergic disease development in offspring has been conducted in a population exposed to a traumatic event during pregnancy (i.e., the study by Turcotte-Tremblay et al., 2014 of women exposed to a severe winter storm). It has been suggested that individuals from war-torn or developing nations are more likely to display altered HPA axis activity due to their experience of multiple and/or chronic traumas and stressors (O’Donnell et al., 2009; Glover et al., 2010). Thus, several lines of reasoning point to selection of women exposed to multiple chronic stressors and/or trauma for the study of PNMS programming of offspring immunity.

As a group, Canadian immigrants are overburdened with poverty and are increasingly part of a visible minority group. Even immigrants who do not experience poverty during resettlement may face significant stress as a result of lowered socioeconomic position (relative to that of their country of origin) due to significant barriers to employment (outlined further in the coming section). Additionally, as a larger portion of Canadian immigrants now arrive from developing nations there may be a greater number of Canadian immigrants that have been exposed to traumatic events in their home country, pre-immigration. This makes Canadian immigrants potentially more likely to exhibit both poor mental health and HPA axis dysregulation in pregnancy. The next sections will
describe the theories and research on immigrant health and mental health, focusing as much as possible on the unique Canadian context and the perinatal period.

1.7 Immigration as a Stressor

Immigration is the relocation to, and resettlement in, a new country. While sometimes referred to as a singular ‘event’, immigration is best conceptualized as a process occurring over an extended period of time that is influenced by multiple factors such as pre-migration experiences, the migration process itself and post-migration experiences (Bhugra, 2004). In Canada, immigrants may be permanent (family class immigrants, economic immigrants, refugees and other permanent residents) or temporary residents (temporary foreign workers, foreign students, humanitarian – including refugee claimants, and other temporary residents) (Citizenship and Immigration Canada, 2013). Regardless of the reason for resettlement, however, immigrants face unique challenges as they adjust to their new surroundings suggesting that immigration is a potential source of significant and persistent life stress (Bhugra, 2004).

Of note, post-migration stressors such as difficulties surrounding employment and finances, displacement from social resources, adjustment to the host country's language and/or culture, and discrimination are among the factors most frequently cited as contributing to the mental health of immigrants (George et al., 2015; Collins et al., 2011; Hansson et al., 2010; Tang et al., 2007; Taniguchi & Baruffi, 2007; Gee et al., 2006; Beiser, 2005; Lerner et al., 2005; Bhugra, 2004; Fenta et al., 2004; Hyman & Dussault, 2000). In addition to stressors unique to the immigrant experience, immigrants are also disproportionately exposed to socioeconomic disadvantage and racial/ethnic discrimination, both of which contribute to stress and poor mental health (Hansson et al., 2010; Beiser, 2005). This is troubling in light of the well-established link between chronic stress and poor physical and mental health (Lupien et al., 2009).
Indeed, research consistently shows that while immigrants are initially ‘healthier’ than their native-born peers, there is a substantial decrease over time in various parameters of immigrants’ health (a phenomenon termed ‘the healthy immigrant effect’ outlined in further detail below). In Canada, immigrants represent a large proportion of the total population and the largest source of population growth. As of 2011, the National Household Survey found that one in five Canadians were foreign-born, making Canada the country with the highest proportion of immigrants among the G8 countries (Chui & Flanders, 2013). From 2001 to 2006, the foreign-born population increased at a rate four times greater than the growth rate of the Canadian-born population (Gushulak et al., 2011). Thus, a better understanding of the unique experiences of immigrants and how these, along with other social determinants of health, contribute to the decline in health of Canadian immigrants is a significant public health concern.

1.7.1 The Healthy Immigrant Effect

A robust finding in developed nations is that of a ‘healthy immigrant effect’ whereby recent immigrants display better health than native-born individuals (even when controlling for age, education and other relevant factors) but then lose this health advantage over time (Kennedy et al., 2006). Canadian research has likewise documented a healthy immigrant effect and subsequent deterioration of various health parameters with increasing years since immigration (Fuller-Thomson et al., 2011; Gushulak et al., 2011; Ng, 2011; Zhao et al., 2010; McDonald & Kennedy, 2004; Newbold & Danforth, 2003; Dunn & Dyck, 2000). The initial health advantage of recent immigrants appears to be due to a self-selection bias (the healthy and wealthy are more likely to immigrate), rigid selection criteria by the host country prior to immigration, and/or healthier behaviours of immigrants before they immigrate (Kennedy et al., 2006). The reasons for the observed decline in immigrant health, however, are more complex and, due to the significant impact on public health, have been the subject of extensive research.
The major theories proposed for the health decline of immigrants with increasing duration of residency in the receiving country are i) convergence, ii) resettlement stress, and iii) the interaction paradigm (Beiser. 2005). Convergence refers to exposure to physical, environmental and/or social factors in the host country that lead to worsening health including both passive exposure (e.g., to pollution) and active exposure (i.e., adoption of the host country's unhealthy behaviours – often called ‘acclimatization’). Resettlement stress refers to the fact that immigrants experience greater exposure to stressors known to be deleterious to health such as poverty and unemployment. The interaction paradigm emphasizes that pre- and post-migration factors interact with personal characteristics and social resources to affect health (Beiser, 2005). Additionally, it has been suggested that the healthy immigrant effect may be due to a reporting bias (resulting from recent immigrants under-reporting health difficulties and/or the emergence over time of previously existing, but undiagnosed, conditions at the time of arrival) (Kennedy et al., 2006; McDonald & Kennedy, 2004) and/or the underuse of preventative health services because of language or income barriers (Kennedy et al., 2006). It is important to note that the mechanisms underlying these models of immigrant health may operate concurrently since the three theories are not mutually exclusive (Llacer et al., 2007).

Particularly concerning, however, is some evidence showing that immigrant health doesn’t just ‘converge to the mean’ (i.e., of the host country’s health levels) but becomes even worse than average levels, a pattern known as ‘immigrant overshoot’ (Urquia et al., 2010; Beiser, 2005). Overall resettlement stress and the interaction paradigm appear to be particularly powerful in explaining the healthy immigrant effect and immigrant overshoot, especially as it pertains to mental health (outlined below). Some studies find that immigrants experience not only higher rates of stressful life events than the native population (Singhammer & Bancila, 2011; Lerner et al., 2005), but also stress that is prolonged or chronic in nature (Lerner et al., 2005). Of note, these stressors are linked with self-reported mental health and immigrants from non-western countries face particularly high rates of these stressors (Singhammer & Bancila, 2011). In Canada immigrants form a large, and growing, part of the total population and given the extensive research showing
immigrants are exposed to myriad different kinds of stressors, it is critical to better understand how these stressors impact the health of this group of Canadians.

1.7.2 Mental Health and Its Determinants in Immigrants

There is conflicting evidence regarding whether immigrants are ‘vulnerable’ or ‘resilient’ in terms of mental health outcomes (Beiser, 2005; Hyman, 2004) since some studies find equal or better mental health in immigrants compared to native-born individuals (Puyat, 2013; Menezes et al., 2011; Breslau et al., 2007; Gee et al., 2006; Ali et al., 2004) while other studies point to poorer mental health in immigrants or immigrant sub-populations (Gannan et al., 2012; Pahwa et al., 2012; Wangel et al., 2012; Collins et al., 2011; Singhammer & Bancila, 2011; Goedhart et al., 2010; Miszkurka et al., 2010; Stewart et al., 2008; Levecque et al., 2007; Dennis & Ross, 2006; Sword et al., 2006; Syed et al., 2006; Lerner et al., 2005; Fenta et al., 2004). Conflicting results are due in part to different measures of mental health (e.g., self-report versus diagnosed mental disorders) and to the fact that immigrants are not a homogeneous group.

The mental health status of immigrants differs by factors common to non-immigrants and by unique immigrant-specific parameters. In other words, the key determinants of health described by the Public Health Agency of Canada (http://www.phac-aspc.gc.ca/ph-sp/determinants/index-eng.php#determinants) operate in immigrant populations. These include income and social status, social support networks, education and literacy, employment and working conditions, social environments, physical environments, personal health practices and coping skills, healthy child development, biology and genetic endowment, health services, gender, and culture. Additionally, there are determinants of health that are specific to the immigrant experience such as time since immigration, region of origin, presence of a language barrier, reason for immigrating, prior experience with Western healthcare system, and degree of acculturation (Beiser, 2005; Hyman, 2004). On an individual level, immigrants also face cultural and social challenges that play a role in mental health outcomes such as cultural conflict and changes to cultural identity (Bhugra &
Jones, 2001). This has led some to classify immigration as a social determinant of health (Castaneda et al., 2015; Fuller-Thomson et al., 2011).

Cross-sectional data from the Canadian Community Health Survey (CCHS) (2009 to 2010 cycle) showed that immigrants, especially recent immigrants (i.e., living in Canada for less than 10 years), displayed a lower prevalence of diagnosed mood and anxiety disorders than Canadian-born individuals (Puyat, 2013). Alternatively, Pahwa et al. (2012), using data from the National Population Health Survey (NPHS) over a 12 year period beginning in 1994/1995, found that longitudinal mental health trends in Canada according to ethnic group painted a different and more complex picture. Their study showed that the predicted probability of moderate/high mental distress was slightly higher for immigrants compared to the Canadian-born population for all ethnic groups except those of Black ethnicity. Other important determinants of mental distress in Canadian immigrants emerging from this study included poor health, low income, and length of stay (described in greater detail below) (Pahwa et al., 2012).

Socioeconomic status (SES) is a salient determinant of health and mental health; however, it may be particularly relevant to the foreign-born population since immigrants, particularly recent immigrants, experience higher rates of unemployment (Yssaad, 2012), and low income (Picot & Hou, 2014) than the Canadian-born population. Some studies have found that differences in mental health outcomes are largely the result of differences in SES between immigrants and the native-born population (Miszkurka et al., 2010; Levecque et al., 2007; Syed et al., 2006). However, others have found that immigrants are more vulnerable to poor mental health when confronted with poverty than those born in the host country (Miszkurka et al., 2012). This could be related to the frustration and disappointment of unemployment and/or under-employment (i.e., where education and/or training are not fully utilized in an individual's job) common to immigrants. For immigrants aged 25 to 54, the rate of unemployment in 2008 was nearly 50% higher than that of the Canadian-born population (6.8% versus 4.6% respectively) (Yssaad, 2012).
Canadian immigrants with a university degree are more likely to be in a low-skilled job than Canadian-born individuals and, moreover, the proportion of immigrants who are underemployed rose from 1991 to 2006 (Galarneau & Morissette, 2008). Unemployment and underemployment negatively impact the mental health of Canadian immigrants through loss of income, social status, employment-related skills (Dean & Wilson, 2009). This additionally impacts physical health as a result of substantial stress for both the individual who experiences the under/unemployment and his/her family members (Dean & Wilson, 2009).

There is a paucity of research on the interaction between sex and other determinants of health in immigrants even though a ‘gender perspective’ has highlighted significant differences in the pre- and post-migration experiences of women compared to men (Llacer et al., 2007). Nevertheless Canadian immigration data have highlighted some important inequalities between the sexes that may impact mental health outcomes. The education of Canadian immigrant women is even less fully utilized than that of immigrant men. Immigrant women with a university degree are even more likely to have a job not requiring it compared to Canadian-born women (Galarneau & Morissette, 2004). Additionally, immigrant women earn less than Canadian-born women (Chui, 2011). It is therefore not surprising that sex differences have been observed in immigrant mental health outcomes that appear to be linked, at least in part, with these SES-related inequalities. A large Canadian study noted an increased risk of mood and anxiety disorders in female immigrants, although only the latter was statistically significant (Patterson et al., 2012). A smaller study of Chinese immigrant women living in Canada have found that negative life events and financial strain related to living below the poverty line were commonly reported and significantly predicted mental health (Tang et al., 2007).

The role of social support and social networks in mental health outcomes is well known; however, there is some research suggesting that, as with SES, an even greater influence may operate in immigrants. Puyat et al. (2013) found that low social support was
associated with a greater likelihood of a diagnosed mood disorder to a greater extent in immigrants, especially recent immigrants (i.e., living in Canada for less than 10 years), compared to Canadian-born individuals. The protective effect of high levels of social support also differed by immigration status with twice as much protection offered in long-term immigrants compared to Canadian-born individuals (Puyat et al., 2013). These results are concerning since Canadian immigrants (especially non-European, less well-established immigrants) are more likely to have poorer social support than Canadian-born individuals (Dunn & Dyck, 2000). The kind of social support received may be important, with emotional support being a particularly salient contributor to mental health outcomes in immigrants (Fenta et al., 2004). Examining the effects of social support on mental health of immigrants in a more indirect way, namely by examining links with neighbourhood density has also demonstrated a powerful effect on mental health outcomes of Canadian immigrants. Linking individual data from the CCHS with neighbourhood-level data from the Canadian Census 2001, Menezes et al. (2011) showed that as the neighbourhood immigrant concentration increases the 12-month prevalence rate of psychiatric disorders in immigrants decreases (with no change in the prevalence rates for non-immigrants).

Time since immigration is an important moderator of mental health in immigrants with increasing time of residence linked with increasing risk of mood disorder (Patterson et al., 2012; Breslau et al., 2007; Gee et al., 2006; Ali et al., 2004; ; Fenta et al., 2004). In Canadian immigrants a lower rate of depression has been reported in recently-arrived immigrants but by 10 years of residence, rates were similar to those of the Canadian-born population (Ali et al., 2004). Longitudinal data from the NPHS showed an inverted U-shaped association between length of stay and mental distress. Immigrants in Canada for less than 2 years and those in Canada for more than 20 years showed lower rates of mental distress than those residing in Canada between 2-20 years (Pahwa et al., 2012). Likewise, a Canadian study of Ethiopian immigrants and refugees found a low risk of developing depression in the first few years after arrival to Canada that increased over time and peaked at 15 years postmigration (Fenta et al., 2004).
As mentioned earlier, the idea of ‘acculturation’ has been put forth to account for the worsening of health over time in immigrants. The process of acculturation involves incorporating new values, attitudes and behaviours and has been called the “conceptual bridge for the relationship between migration and changes in health” (Hyman & Dussault, 2000, page 357). However, research on acculturation is complicated by differing conceptualization and definitions between studies. Some authors have used a single variable, such as language barrier or length of stay, as a proxy for acculturation, while others have used validated acculturation scales or questionnaires. A related concept is that of ethnic identity. For example, one Canadian study found that in Ethiopian immigrants a stronger Ethiopian identity was associated with a lower rate of depression (Fenta et al., 2004). Alternatively, it has been suggested that greater exposure to discrimination may occur with increasing length of residency and this may explain, at least in part, the poorer mental health of immigrants over time (Gee et al., 2006) since various forms of discrimination contribute to poor mental health (Yip et al., 2008; Gee et al., 2006; Noh & Kaspar, 2003; Liebkind & Jasinskaja-Lahti, 2000).

Region of origin also moderates immigrants’ risk for mood disorders (Pahwa et al., 2012; Patterson et al., 2012; Singhammer & Bancila, 2011; Miszkurka et al., 2010; Ali et al., 2004) possibly due to genetic or cultural differences. To complicate matters further, there can be interactions between these determinants that lead to different outcomes with respect to rates of mood disorders. For example, time since immigration and socioeconomic status demonstrated differential outcomes for men and women in a Canadian study whereby the lowest rate of depression was seen in low-income recent male immigrants while the highest rate of depression was in low-income non-recent female immigrants (Smith et al., 2007). Pahwa et al. (2012) found differences in the likelihood of mental distress over time only in some Canadian immigrant ethnic groups. Specifically, immigrants of Black ethnicity were less likely to report mental distress than other ethnicities initially but by a decade
later they had a higher probability of mental distress compared to other ethnicities (Pahwa et al., 2012).

Thus determinants of mental health in immigrants are numerous, interactive, and in some cases their effects may be non-linear. Prior research has shown that separate components of ‘SES’ (namely poverty, employment, and education), social support, time since immigration, language barrier (as a proxy for acculturation), and region of origin are particularly salient determinants of mental health for Canadian immigrants. Research studies examining immigrant mental health must include the measurement of these determinants to truly understand how immigration-related factors impact health outcomes.

### 1.7.3 Mental Health of Pregnant Immigrant Women

Pregnant immigrant women display elevated rates of stress and/or depression in most (Balestrieri et al., 2012; Giardinelli et al., 2012; Goedhart et al., 2010; Miszkurka et al. 2010; Zelkowitz et al., 2008; Huang et al., 2007; Taniguchi & Baruffi, 2007; Zelkowitz et al., 2004; Hyman & Dussault, 2000) but not all studies (Dhillon & MacArthur, 2010; Huang et al., 2007). This appears to result, at least in part, from the higher rates of known risk factors for antenatal depression such as life stress and poor social support (Dudas et al., 2012; Yonkers et al., 2011; Lancaster et al., 2010; Melville et al., 2010; Lusskin et al., 2007; Rubertsson et al., 2005) in immigrants (as described in the previous section). Other predictors of perinatal depressive symptoms such as a language barrier and unemployment (Rubertsson et al., 2005) are also common to immigrants, placing them at a higher risk. This suggests a clustering of risk factors for prenatal psychological distress occurs in immigrant women. For example, in a Swedish study, pregnant women with a language barrier were more likely to have financial problems and a lack of social support, and these women also had more depressive and post-traumatic stress symptoms during pregnancy (Wangel et al., 2012).
This compounding risk has been demonstrated in Canada as well. In the largest prospective study of the predictors of prenatal depression in immigrant and non-immigrant women, Miszkurka et al. (2012) examined 5,162 pregnant women living in Montreal, of whom 1,400 were born outside of Canada. They found that pregnant immigrant women were more often exposed to high marital strain, lack of social support, lack of money, and crowded living conditions compared to Canadian-born women, independent of time since immigration. Furthermore, compared with Canadian-born women, immigrant women were more prone to antenatal depression when faced with lack of money for basic needs. Canadian research has likewise found that in the early postpartum period immigrant women were more likely to have low income, low social support, and to need financial assistance (Sword et al., 2006). This suggests that pregnant immigrant women living in Canada experience more psychosocial and life stressors and are especially vulnerable to perinatal depression.

High rates of prenatal stress and depression are also troubling because they are significant predictors of postpartum depression (PPD) (Giardinelli et al., 2012; Martini et al., 2010; Klainin & Arthur, 2009; Milgrom et al., 2008; Heron et al., 2004; Robertson et al., 2004) which has myriad negative and far-reaching consequences for both maternal and child health. The rate of postnatal depression in immigrants and refugees is reportedly four times higher than that of native-born women (Collins et al., 2011). In at least one Australian study, elevated postpartum depressive symptoms in non-English speaking immigrant women were observed even when controlling for SES variables (Lansakara et al., 2010). Of particular concern, compared to Australian-born women, these new mothers were also less likely to be asked about emotional health and relationship problems despite similar rates of contact with health care providers. Canadian studies have similarly reported that immigrant women are more likely to be at risk for postpartum depression, and are also less likely to receive help and support for emotional problems (Gannan et al., 2012; Sword et al., 2006). Furthermore, foreign-born women may also be less likely to talk
to a health care professional about clinically significant prenatal depressive symptoms (Huang et al., 2007) perhaps due to the multiple obstacles they face in receiving PPD treatment including practical (e.g., language barrier) and culturally-related barriers such as stigma (O’Mahony & Donnelly, 2010), the latter being a particularly understudied area (Bina, 2008). This applies in pregnancy as well, since pregnant women with a language barrier are less likely to consult with a psychiatrist or psychologist (Wangel et al., 2012). Clearly more research on perinatal depression in immigrants and refugees is needed to inform better clinical practice.

Ethnicity and/or country of origin may have a substantial impact on PNMS and/or prenatal depression. The prevalence rate for prenatal depression varies considerably between nations; some have reported rates similar to those of Westernized countries while others report substantially higher rates. In Africa and Italy, for example, antenatal depression is reportedly present in 11% and 8-20% of pregnant women respectively (depending on cut-off values used) (Balestrieri et al., 2012; Giardinelli et al., 2012; Sawyer et al., 2010); however, much higher rates have been found in Pakistan (26%), China (29%); and Jordan (19-57%) (Zeng et al., 2015; Abujilban et al., 2014; Husain et al., 2011; Mohammad et al., 2011). Furthermore, these international studies as well as studies of different ethnic groups living in Westernized countries have demonstrated some unique risk factors for antenatal depression such as conflict with mother-in-law in Middle Eastern women (Mohammad et al., 2011) and the family’s (but not the mother’s) preference for a male baby in Asian women (Dhillon & MacArthur, 2010).

With the realization that mental health during pregnancy may differ by ethnicity there is growing interest in the rates and risk factors for antenatal depression in women from non-Western backgrounds living in Westernized countries. In the U.S. there is some evidence that ethnicity may be a more significant contributor to prenatal depression than country of birth. For example, non-white ethnicity (i.e., Asian, African American, and Hispanic ethnicity) has been identified as a risk factor for antenatal depression (Melville et al.,
2010), even after adjustment for various risk factors (Gavin et al., 2011); however, other research has shown that in most ethnic groups U.S.-born women had a higher prevalence of prenatal depressive symptoms than foreign-born women (except in Asian women) (Huang et al., 2007). A different pattern by region of origin was seen in a recent Norwegian study where women from the Middle East and South Asia had significantly higher risk for prenatal depression compared to other ethnicities and Western Europeans even after adjustment for SES and social support variables (Shakeel et al., 2015).

These studies reflect the socio-cultural climate of the host country, however, which is substantially different than that of Canada. In Canada, large differences in the rates of prenatal depression by region of origin have been reported. Miszkurka et al. (2010) found that women from Europe or the Middle East had a rate of prenatal depression similar to those of Canadian-born women while women from the Caribbean, the Maghreb and Sub-Saharan Africa had double the rate of prenatal depression than Canadian-born women. Socioeconomic factors and social support accounted for a majority of these elevated odds (Miszkurka et al., 2010). Looking at these data more closely, time since immigration appears to play an important role although this differs by region of origin. As a group, immigrant women are at higher risk of prenatal depression independent of time since immigration; however, for different groups of immigrants depressive symptoms increased, decreased, or fluctuated with length of stay (Miszkurka et al., 2010). Risk factors for prenatal depression also differ by time since immigration. Another study by this group found that long-term immigrants (living in Canada for 9 years or more) were most exposed to lack of money for basic needs during pregnancy while newly arrived immigrants (living in Canada for up to 2 years) were most exposed to adverse life events (Miszkurka et al., 2012). Similarly, in a small qualitative Canadian study of pregnant South East Asian immigrant women, those who were more acculturated (i.e., longer time since arrival and fluent in English and/or French) were more likely to report a great deal of stress compared to those who were less acculturated (Hyman & Dussault, 2000). Sources of stress mentioned most often in the more acculturated immigrants were related to financial pressures, work stress, and a lack of social support.
Canada has the highest per capita immigration rate in the world (Canada's Immigration Program, 2004), with immigrants comprising 20% of the Canadian population (Statistics Canada accessed at www.statscan.gc.ca). Given the research outlined above, chronic stress and depression in pregnant immigrant women is a significant public health concern in Canada, as it is in other developed countries. Furthermore there is a need for more research that examines not just rates and determinants of stress/depression in these women, but also the consequences on maternal and child health. Key contributors to mental health in pregnant immigrant women have been identified (region of origin, time since immigration, financial difficulties, lack of social support), however, the mechanisms by which immigration-related stress may affect health in pregnancy and subsequent health of children are not well understood and warrant further investigation.

### 1.7.4 Consequences of Prenatal Stress for Maternal and Infant Health in Immigrants

In a systematic review of perinatal health in migrants to western industrialized countries (the U.S., Europe, Australia, and Canada), Gagnon et al. (2009) found that in over 50% of studies examined migrants displayed better birth outcomes (lower rates of preterm birth, PTB, and low birthweight, LBW) and higher rates of health-promoting behaviours compared to receiving-country women. The finding that immigrants have better or equally good birth outcomes compared to native-born women despite their lower SES and less use of prenatal care has been dubbed the ‘epidemiological paradox’ (Beiser, 2005), and is thought to result from better maternal health and healthier behaviours during pregnancy. This epidemiological paradox has not been universally reported, however. For example, another systematic review that compared pregnancy outcomes of immigrant and non-immigrants across European studies provides a sharp contrast with the finding that immigrant women had worse outcomes including an increased risk of LBW, PTB, perinatal mortality, and congenital malformations (Bollini et al. 2009). The discrepancy between these two systematic reviews is likely due to the different outcomes examined and the
different countries included in the reviews. Of particular interest, however, the review by Bollini et al. (2009) also found that receiving countries with strong integration policies had significantly reduced risks of poor outcomes for immigrant women. The authors proposed that the lowered risks may operate through reduced stress and discrimination for immigrant women in countries that promote inclusion of immigrants in the receiving country’s society. Thus, while not directly measuring stress, these findings implicate stress in poor outcomes for immigrant mothers and their children.

Research in Canadian immigrant women has demonstrated various moderators of birth outcomes in this group including socioeconomic status, country of origin (Urquia et al., 2009) and length of residency. Specifically, low levels of education (Auger et al., 2008) and increasing duration of residency (Urquia et al., 2010; Ray et al., 2007) have been associated with poor birth outcomes (e.g., PTB, LBW, small for gestational age infants and pre-eclampsia). Increased rates of smoking and pre-existing medical conditions such as hypertension, obesity, and asthma appear to explain, at least in part, the association between poorer birth outcomes and length of time since immigration (Urquia et al., 2012; Ray et al., 2007).

Less explored predictors of perinatal outcomes in immigrant women include language ability (Gagnon et al., 2009) or acculturation and stress. Furthermore, few studies have examined physiologic mechanisms that may underlie the connection between prenatal distress and child health in immigrant women. In a study of women of Mexican descent in the U.S., D’Anna et al. (2012) found that more acculturation was associated with poorer birth outcomes and also with a flatter diurnal slope in late pregnancy. As outlined in previous sections, prenatal HPA axis activity is implicated in many negative pregnancy and birth outcomes as well as adverse long-term developmental outcomes in the child. The novel finding that cortisol mediated the effect of acculturation on lower infant birth weight is intriguing and suggests more research on the physiological correlates of immigration-related factors are warranted.
Studies outside of Canada have found that the association between prenatal stress/depression and poor birth outcomes in immigrants may be moderated by social support (particularly paternal support) (Ghosh et al., 2010) and region of origin (Goedhart et al., 2010). Thus, there may be some important protective factors within certain cultures/ethnicities that buffer women (and their children) from the ill effects of prenatal distress. Alternatively, some potent determinants of child health, such as poverty (Larson, 2007), disproportionately affect Canadian immigrant women and children (Seguin et al., 2012) and may therefore make a greater contribution to poor health and/or render this portion of the Canadian population more vulnerable to disease.

Over a decade ago, after reviewing the evidence on Canadian immigrants’ health, Hyman (2004) proposed the need for more research on immigrant subgroups with specific needs and research on acculturation-related changes including stress. This need still exists today, particularly for pregnant immigrant women and their children given the clustering of stressors in this group and the far-reaching consequences of prenatal stress on infant and child health (described in previous sections). Additionally, exploration of the mechanism through which social determinants (e.g., poverty) and immigration-related factors (i.e., region of origin, time since immigration and acculturation) contribute to ill health in both mother and child is vital to our understanding of immigrant health.

1.8 Study Aims, Objectives, and Hypotheses

The aim of this pilot study is to determine the feasibility of utilizing a rigorous, prospective methodology in an immigrant population to examine the association between prenatal maternal stress and atopic outcomes in offspring.

As outlined in section 1.4.3, the most important moderators of the association between PNMS and atopic outcomes in offspring in non-immigrant populations are infant age,
maternal atopy, and the sex of the offspring. As the present study is a pilot with a small sample size, rather than stratify analyses, analyses will be adjusted for these moderators. Key factors that contribute to the mental health of pregnant immigrant women and modulate its association with birth outcomes have been identified (summarized in Sections 1.7.3 and 1.7.4). In Canada these include especially SES, social support, country of origin, time since immigration, and acculturation/language barrier; however, the extent to which these factors play a role in contributing to allergic outcomes has never been explored.

Figure 1 outlines the model, created by integrating prior research, which will be used as a framework for the study’s primary objective and first secondary objective. Given that potential mechanism(s) underlying the link between PNMS and atopic outcomes in offspring have been previously suggested but remain unsupported by data, a second model (Figure 2) was created to provide the framework for exploring these in the second secondary objective.

Specifically, the primary objective of the present study is:

i) To examine whether prenatal stress is associated with susceptibility to allergic disease in offspring of a multi-ethnic, community sample of Canadian immigrant women

The secondary objectives of the present study are:

i) To examine the psychosocial and immigration-related factors that modulate this association.

ii) To examine possible mechanisms underlying this association including:
   a. Altered maternal prenatal HPA axis activity
   b. Altered infant HPA axis activity
   c. Altered prenatal maternal immune parameters

We hypothesize that:

1) Prenatal stress will be positively associated with susceptibility to allergic disease in the offspring.

2) Psychosocial and immigration-related factors will mediate this association.
3) Alterations in maternal prenatal HPA axis activity and prenatal maternal immune parameters will be found in association with prenatal maternal stress and/or significant mediating variables.

Figure 1: Mediators and Moderators of Prenatal Maternal Stress Programming of Infant Allergy

*Determinants of health were examined as possible mediators; moderators were used as covariates
Previous research has shown that pregnant immigrant women experience higher rates of stress and depression than the native-born population. Prenatal stress is associated with changes in maternal stress hormones and immune parameters, and these changes are thought to potentially 'program' offspring development thereby conferring disease risk in later life. In particular, there is evidence suggesting that prenatal stress may program immune development in the offspring toward a greater susceptibility to allergic disease. To date, no studies have prospectively examined whether stress is associated with

Figure 2: Pathways to Prenatal Maternal Stress Programming of Infant Allergy

**Summary**

Previous research has shown that pregnant immigrant women experience higher rates of stress and depression than the native-born population. Prenatal stress is associated with changes in maternal stress hormones and immune parameters, and these changes are thought to potentially 'program' offspring development thereby conferring disease risk in later life. In particular, there is evidence suggesting that prenatal stress may program immune development in the offspring toward a greater susceptibility to allergic disease. To date, no studies have prospectively examined whether stress is associated with
infant/childhood allergic outcomes in an immigrant population. Furthermore, there is a
dearth of studies that examine altered stress or immune parameters in pregnant immigrant
women. Prior research has shown that health outcomes in Canadian immigrants are
influenced by both immigration-specific and non-specific determinants of health. As such,
the possible mediating or modulating role of these factors cannot be ignored in health
research involving pregnant Canadian immigrants.

As Dunkel Schetter (2011) explained:

...past research on pregnancy has illustrated that without interdisciplinary study,
the ability to understand and improve the outcome of mothers, their offspring, and
their families will be limited (page 547).

This study integrates research from psychiatry, psychology, sociology, endocrinology, and
immunology in an attempt to understand the correlates and consequences of prenatal
stress, specifically with respect to possible programming of offspring immunity, in an
immigrant population.

To date no Canadian studies have been conducted on a multi-ethnic, multi-lingual,
community-based sample of pregnant immigrant women whereby psychologic and
physiologic measures of stress are collected. This is therefore a pilot study to determine
the feasibility of achieving the above-stated objectives, with the overarching goal of
understanding the barriers and facilitators to conducting this type of research in the
Canadian context. Pregnant Canadian immigrant women must be included in research
studies if we are to gain an accurate picture of the inter-generational transmission of health
and/or disease vulnerability in Canada. This is not an easy or inexpensive task however
the reward is the promotion of better health for all Canadians.
2 Methods

2.1 Study Design, Community Collaborations & Translation of Study Materials

As part of the study design, and prior to participant recruitment, a partnership was established between study investigators and the St. Joseph’s Immigrant Women Centre ‘Facilitating Inclusion Co-operative’ or FIC (Hamilton, Canada). Consultation with FIC’s program leader resulted in the selection of several “community-based researchers” (CBRs). The CBRs were women who were: i) themselves immigrants to Canada, ii) fluent in English and at least one other language, iii) knowledgeable of and/or have received training in basic medical terminology and research methodology, and iv) connected with immigrant and/or ethnic communities in the Hamilton and surrounding area. Two meetings were conducted with the study coordinator (the author), the FIC program leader and the CBRs to discuss potential issues with the study design, to brainstorm ideas for maximizing participant recruitment, and to initiate translation of relevant study materials. These initial meetings resulted in the selection of two ethnic groups from within the Hamilton community, for the present study’s sample: i) Chinese-speaking (including both Mandarin and Cantonese) and ii) Arabic-speaking immigrant women. These two groups were chosen based on the abundance of these immigrants in the Hamilton area and the availability of more than one translator via the FIC for translation and back-translation of study materials.

Two subsequent group meetings were held to discuss possible cultural issues that might interfere with participant recruitment and/or data collection, possible participant recruitment sites, and the design of study advertisements. Two CBRs (one Arabic-speaking and one Chinese-speaking) were chosen for translation of study flyers and relevant study materials: the information and consent form, saliva collection instructions and diary, and a key study questionnaire - the Crisis in Family Systems-Revised (CRISYS-R). Two different CBRs (one for each language) were then selected for blinded back-translation of the study materials listed above. During both translation processes CBRs were encouraged to seek clarification from the study coordinator about the materials as needed. They were further
instructed to record any issues arising during translation and/or any changes to wording they felt were necessary, along with their reasoning or justification for these changes. The study coordinator then reviewed the back-translated documents for clarity and flow and discussed any issues identified during the translation process with the CBRs involved. Overall there was excellent agreement between the original documents and the back-translated versions with only minor issues (e.g., use of a slang word for ‘spit’ rather than clinical terms such as ‘saliva’).

2.2 Participant Recruitment

Stage 1

One CBR from each ethnic group was then chosen as the primary translator for the duration of the study and she consented to the use of her name and telephone number on study recruitment posters and flyers. All CBRs were asked to distribute both English and translated versions of study flyers at various community locations, including community centres, churches, and ethnic food stores. CBRs were also encouraged to spread information about the research study by word of mouth. CBRs were paid for their time and compensated for parking and mileage accrued during meetings and participant recruitment.

CBRs were given a printed list of study recruitment criteria (i.e., less than 12 weeks gestation and born outside of Canada) along with a basic outline of the study design (number of study visits involved, type of tests conducted during as part of study participation, compensation provided, etc.). CBRs were also given a short data collection form which they were asked to fill out when meeting any potential participants, detailing the participant’s full name, telephone number, current gestational age, expected due date, country of origin, English fluency (yes/no), medical history, current medication use, and the need for childcare during study visits (yes/no). The study coordinator and the CBRs reviewed the information on these data collection forms by phone for every potential
participant and those who fit the study inclusion criteria were enrolled in the present study. The study coordinator called the participants who did not require any translation to enroll them in the study and to arrange the date and time for the first study visit. For participants requiring translation the CBR acted as the liaison between the study coordinator and participant, enrolling them and arranging the first study visit (which the CBR would also be attending).

Stage 2

After approximately three months of active recruitment, the rate of recruitment was deemed too slow for timely completion of the study. Subsequent meetings were then held with CBRs from two other ethnic groups: Spanish-speaking and Urdu-speaking (including also other commonly-spoken South Asian languages, namely Punjabi and Hindi). Again, relevant study materials were translated and back-translated by separate, blinded CBRs and any issues with these translations were screened by the study coordinator and discussed with appropriate CBRs as needed. At this time another study inclusion criterion was also broadened: ‘women at less than 12 weeks gestation’ was increased to ‘less than 20 weeks gestation’.

Stage 3

After two more months, recruitment was improving but still slow. Therefore, final changes to participant inclusion criteria were made as follows: i) any woman born outside of Canada and ii) at less than 28 weeks gestation at study enrollment. Additionally, further meetings were held with key staff members of the North Hamilton Community Health Centre, the Urban Core Community Health Centre, and midwifery clinics (Hamilton Midwives, Community Midwives of Hamilton, and Access Midwives: Stony Creek) to inform them of the study details, participant inclusion criteria, and relevant contacts (i.e., CBRs and the study coordinator). Study flyers were distributed and posted on bulletin boards at these locations and key contacts, specifically midwives, were given ‘contact cards’ that
could be kept at their desks with study inclusion criteria and contact information for the study coordinator and primary CBRs for each language.

The original study sample size was 60; however, the changes to inclusion criteria outlined above resulted in greater ethnic variability than previously expected. Consultation with the student’s primary supervisor (Dr. M. Steiner) and other committee members led to the decision to increase the sample size to a minimum of 75 to account for the extra variability.

2.3 Subjects

Pregnant women who were 18 years or older, born outside of Canada and less than 28 weeks gestation were invited to participate in the present study. Participants with current post-traumatic stress disorder (PTSD), as assessed by the Harvard Trauma Questionnaire (HTQ) (Mollica et al., 1992) at the initial study visit, were to be excluded; however, none of the participants were excluded based on this criterion.

2.4 Study Visits & Data Collection

Overview

The study was approved by the St. Joseph’s Healthcare Hamilton Research Ethics Review Board. Verbal and written consent were obtained for each participant. Participants were followed prospectively from early- to mid-pregnancy to a minimum of one year postpartum. Data were collected at four time points in total: i) study visit #1, at less than 28 weeks gestation, ii) study visit #2, at 32 to 40 weeks gestation, iii) chart review of infant birth record, and iv) study visit #3, at a minimum of one year postpartum. At each of the three study visits participants were provided with a) access to free childcare for the duration of the visit, b) the option of a translator for the duration of the visit, c) financial
reimbursement, and d) transportation-related reimbursement (either two bus tickets or a 4-hour hospital parking pass).

Visit #1 (< 28 weeks gestation)

Participants were asked to refrain from the use of certain medications which are contra-indicated for skin-prick testing, namely anti-histamines, sleep aids and over-the-counter cough and cold remedies for four days prior to the first study visit. The study coordinator and CBRs were given a list of the generic names of these medications to cross-reference any potentially contra-indicated substances mentioned by participants. Participants were asked to notify the study coordinator or CBR if they began taking any of these medications in order to cancel and re-schedule the visit. Participants were also reminded of this and queried again about the use of these medications the day prior to the visit and upon arrival to the clinic before the interview (described below) began.

Participants were interviewed by the study coordinator, or with the aid of a CBR as needed, in a private room at the Women’s Health Concerns Clinic, St. Joseph’s Healthcare Hamilton. Interviews lasting between 2 and 3 hours were used to collect information about maternal demographics and health, immigration-related factors, and characteristics of the home environment. Participants were also assessed via standardized questionnaires with respect to current psychiatric status (Primary Care Evaluation of Mental Disorders Patient Health Questionnaire [PRIME-MD PHQ] and Harvard Trauma Questionnaire [HTQ]), stress (Crisis in Family Systems – Revised [CRISYS-R], Perceived Stress Scale-10 [PSS-10]), depressive symptoms (Edinburgh Postnatal Depression Scale [EPDS]), and social support (Multidimensional Scale of Perceived Social Support [MSPSS]) (described in greater detail in section 2.5). Additionally, participants underwent skin-prick testing by a certified respiratory technician for assessment of atopic status (detailed in section 2.6.3). Finally, participants were provided with a salivary sampling kit for collection of saliva samples by passive drool at six time points, across two consecutive days, in their own homes. The
protocol for salivary sampling (detailed in section 2.6.1) was also verbally explained at this
time and participants were encouraged to seek clarification about the process of saliva
collection. All participants were given written instructions for the saliva sampling protocol
in the language of their choice (provided it was one of English, Chinese, Spanish, Arabic, or
Hindi-Urdu). At the end of the study visit, participants received financial compensation
($30) and transportation reimbursement.

Visit #2 (32-40 weeks gestation)

Participants were interviewed by the study coordinator or with the aid of a CBR as needed
in the same location as the first visit. Interviews lasting approximately 1 hour were used to
collect information about maternal health including any pregnancy-related complications,
change in medication use, expected date of delivery (to confirm gestational age), hospital of
expected delivery, changes to the home environment since Visit #1, and self-reported
changes in sleep, appetite and mood. Participants were again administered standardized
questionnaires for assessment of current stress (CRISYS-R, PSS-10), depressive symptoms
(EPDS), and perceived social support (MSPSS). Salivary sampling kits were given to
participants, using the same protocol as in Visit #1. Finally, venipuncture by a registered
nurse was conducted for collection of blood samples to be analyzed for both clinical and
research purposes (see section 2.6.2). Upon completion of the study visit, participants
received financial compensation ($30) and travel-related reimbursement.

Data Collection from Hospital Birth Records

Information about the delivery and the infant’s birth characteristics were collected from
the baby’s hospital chart including mode of delivery (vaginal or caesarian section), use of
epidural during delivery (yes/no), and the infant’s sex, gestational age, birth weight, length,
Apgar scores at 1- and 5-minutes, and head circumference. Charts were also examined for
information about maternal medication use during pregnancy, pregnancy complications
and psychosocial issues to confirm accuracy of information collected during study interviews.

Visit #3 (at >1 year postpartum)

For the final study visit, at a minimum of 1 year postpartum, participants came with their infants for assessment by the study coordinator (and CBR, as needed). A semi-structured interview was conducted to collect information on maternal and infant health since the infant’s birth. Mothers were administered the EPDS, PSS, and MSPSS, the CRISYS-R (this time, unlike during other study visits, the CRISYS-R encompassed the time since birth not just the 6 months prior), and the Resiliency Scale (RS). Salivary sampling kits were given to participants for collection of saliva according to the same protocol as in previous visits. Participants were also provided with a salivary sampling kit for their infant and given verbal and written instructions on infant saliva sampling protocol (detailed in section 2.6.1) in the language of their choice. Finally, infant skin-prick testing was performed by a respiratory technician for assessment of the infant’s atopic status (detailed in section 2.6.3). Clinical history of a physician-diagnosed allergy (including eczema) or the occurrence of atopic dermatitis/food allergy (defined as skin rash, respiratory or abdominal symptoms within 4 hours of ingestion of a particular food, on 2 separate occasions) was assessed via maternal report.

2.5 Questionnaires

WHCC Intake Form (Demographics and Health-Related Information)

The Women’s Health Concerns Clinic (WHCC) intake form was administered to gather information on maternal characteristics, including medical and psychiatric history (diagnoses, treatment and use of psychotropic medication), current use of medications and vitamins, marital status, education level, current occupation and work/school status, partner’s age and current occupation/work/school status, reproductive history (age at
menarche, contraceptive history, number of previous pregnancies, number of children, miscarriages, date of last menstrual period, expected due date of the current pregnancy), familial psychiatric history, current and past cigarette, alcohol and illegal substance use, and current life stressors. More specifically, maternal psychiatric history was assessed by asking women if they had ever been diagnosed with or treated/counselling for a mood or anxiety disorder by a health professional. This questionnaire was administered at visit #1 only.

**Immigration-Related Information**

Information pertaining to the immigration process was collected by semi-structured interview to assess participants’ country of origin, date of immigration to Canada (to calculate number of months since immigration), reason(s) for immigrating (qualitative, descriptive), the type and number of persons immigrating with the participant, and a life history relating to immigration (e.g., migration to other countries prior to immigration to Canada). This questionnaire was administered at visit #1 only.

**Allergenic Load of the Home Environment**

Information relating to the ‘allergenic load’ of the participants’ home environment was gathered including the presence of pets, approximate percentage of carpeting/rugs (less than 50% or more than 50%), use of humidifier (yes/no), and frequency of incense burning. Participants were also asked whether their partners (i.e., the baby’s father) currently smoked cigarettes and if so, whether this ever occurred in the home. This questionnaire was administered at visit #1 only; however, at visits #2 and #3 mothers were asked whether there were any changes since the last visit to any of their answers regarding pets, carpets, humidifier or incense use.

**Maternal, Paternal, and Infant Allergic History**
Maternal, paternal, and infant allergic history were assessed by asking mothers about allergic diagnoses and symptoms experienced by her, the baby’s father (both at visit #1) and the infant (at visit #3). Specifically: i) for allergic rhinitis: “Have you (or your partner or infant) ever been diagnosed with allergic rhinitis?” and “Did you (or your partner or infant) ever have a runny nose/itchy or watery eyes when you did not have a cold/flu?”, ii) for asthma: “Have you (or your partner or infant) ever been diagnosed with asthma?” and “Did you (or your partner or infant) ever have trouble breathing or have a cough/wheeze when you did not have a cold/flu?”, iii) for eczema: “Have you (or your partner or infant) ever been diagnosed with eczema?” and “Did you (or your partner or infant) ever have a rash/hives/itchy bumps on your skin?”, iv) for other diagnosed allergies (i.e., to foods, medication, animals etc.): “Have you (or your partner or infant) ever been diagnosed with any other allergies?”.

Previous longitudinal research has shown that an early diagnosis of eczema (i.e., atopic dermatitis between 1-3 years of age) is a strong predictor of allergic disease (atopic dermatitis, asthma, and rhinoconjuctivitis) at age 6, 7, and 10 (Kim et al., 2013; Kjaer et al., 2009; Kurukulaaratchy et al., 2005; Gustafsson et al., 2000). While there is a contribution of both parents to the risk of allergic disease development, the maternal contribution is particularly robust (Soto-Quiros et al., 2002). Thus, responses were used to establish a history of maternal allergy and infant allergic disease, as diagnosed by a doctor.

**Harvard Trauma Questionnaire (HTQ)**

Previous exposure to traumatic events and current PTSD symptoms were assessed using the Harvard Trauma Questionnaire (HTQ) (Mollica et al., 1992). The HTQ is a simple and reliable screening instrument that identifies symptoms related to immigrant and refugee experiences which are associated with PTSD criteria. The HTQ is composed of 16 items focusing on symptoms experienced in the past week, rated on a scale of 1 (not at all) to 4 (extremely). The final score is the sum of all item ratings divided by 16, and a score greater
than 2.5 is considered representative of an individual who is symptomatic for PTSD. The HTQ is well received by refugee patients and bicultural staff and is culturally sensitive, making it especially useful in the assessment of highly traumatized non-Western populations (Mollica et al., 1992). This questionnaire was administered at visit #1 only. As post-traumatic stress disorder has been shown to have marked associations with altered HPA axis activity that transfers to subsequent generations (Yehuda et al., 2005), current PTSD (i.e., HTQ score > 2.5) was an exclusion criterion for the present study.

**Primary Care Evaluation of Mental Disorders Patient Health Questionnaire (PRIME-MD PHQ)**

The Primary Care Evaluation of Mental Disorders Patient Health Questionnaire (PRIME-MD PHQ or simply, PHQ) was used to screen for the presence of current major psychiatric disorders (Spitzer et al., 2000). The PRIME-MD PHQ is a quick tool for assessment of psychiatric morbidity that contains items corresponding to DSM-IV diagnoses and demonstrates good agreement with mental health professionals’ diagnoses (Spitzer et al., 1999). Furthermore, the PHQ has been well-validated in an obstetric population (Spitzer et al., 2000). More specifically, the PHQ assesses mood disorders (including major depressive disorder), anxiety disorders, probable alcohol abuse/dependence, and eating disorders. This questionnaire was administered at visit #1 only.

**Edinburgh Postnatal Depressive Scale (EPDS)**

Participants were assessed for depressive symptoms with the Edinburgh Postnatal Depressive Scale (EPDS) (Cox et al., 1987). The EPDS assesses mood symptoms in the past 7 days with 10 items rated on a 4-point scale where higher scores indicate greater depressive symptoms. Various cut-off scores have been used in clinical and research settings, while many other studies utilize the scale as a continuous measure. For the present study, any participant scoring 11 or more on the EPDS was offered counseling with a WHCC clinician. The EPDS has been widely used to assess depressive symptoms during
pregnancy and the postpartum period and exhibits high sensitivity and specificity to depression in relation to standardized psychiatric interviews (Cox et al., 1987). Of interest, the EPDS contains distinct (but correlated) anxiety and depression subscales (Jomeen & Martin, 2005), although some research has shown the total EPDS score (rather than the anxiety subscale *per se*) more closely measures anxiety (Brouwers et al., 2001). The EPDS has been translated into numerous languages and the published, translated version was administered in participants’ native language as needed. This questionnaire was administered at visits #1, #2, and #3.

**Crisis in Family Systems-Revised (CRISYS-R)**

‘Life stress’ was assessed with the Crisis in Family Systems-Revised (CRISYS-R), a 63-item instrument that examines the occurrence of life stressors in the previous 6 months (Shalowitz et al., 1998). Items are divided into eleven domains: financial, legal, career, relationships, safety in the home, safety in the community, medical issues pertaining to self and others, home issues, authority, and prejudice. Each positively endorsed item is assessed along 2 dimensions: valence (positive, negative, neutral), and chronicity (resolved, ongoing) allowing for a comprehensive picture of 'life stress' (Shalowitz et al., 1998). The CRISYS-R was designed for use in a low-income, urban population and therefore captures many stressful life events pertinent to the immigrant experience. For example, the domain of prejudice reflects exposure to racism, a stressor known to contribute to poor health in immigrant women (Guruge et al., 2009). Furthermore, the CRISYS-R has been translated into Spanish and found to be a valid and reliable measure of life stressors in a Spanish-speaking population living in the U.S. (Berry et al., 2006). Of particular interest, the CRISYS-R scale has been used as part of the Asthma Coalition on Community, Environment and Social Stress (ACCESS) cohort to investigate associations between maternal (prenatal) psychosocial variables and atopic outcomes in the infant (see Appendix A). This questionnaire was administered at visits #1, #2, and #3.
Scores on the CRISYS-R were used to create two measures of interest: stressful life events and financial hardship. To conceptualize the extent of stressful life events a ‘CRISYS-R-neg’ score was created by summing the number of items endorsed as negative, as others have done (Suglia et al., 2010). This was done for CRISYS-R scores at both visit #1 and visit #2. The experience of financial hardship was defined as endorsing one or more stressful life event from the financial domain and appraising it as negative. This was done separately for responses to the CRISYS-R-neg scale at visits #1 and #2. The financial domain consists of relatively severe stressors such as gas or electricity shut-offs, affordability of basic resources such as clothing and therefore endorsing any of these SLEs can be viewed as a proxy for low SES and/or poverty.

**Perceived Stress Scale-10 (PSS-10)**

Level of perceived stress was measured with the Perceived Stress Scale (PSS-10), a 10-item questionnaire designed to measure appraised/experienced stress (Cohen et al., 1983). Items query feelings and thoughts in the past month, rated on a scale from 0 (never) to 4 (very often). Total scores range from 0-40 with higher scores indicating greater perceived stress. The PSS has been translated into a number of different languages and used in immigrant and refugee populations (Gagnon et al., 2004). This questionnaire was administered at visits #1, #2, and #3.

**Multidimensional Scale of Perceived Social Support (MSPSS)**

Level of perceived social support was assessed with the Multidimensional Scale of Perceived Social Support (MSPSS), a 12-item questionnaire that assesses emotional help and satisfaction with social support networks, specifically family and friends (Zimet et al., 1988). Items are rated on a 7-point scale with higher scores indicating greater perceived social support. This scale has been translated into Russian and used previously in studies examining mental health outcomes in immigrant populations (Ritsner & Ponizovsky, 2003). This questionnaire was administered at visits #1, #2, and #3.
Resilience Scale (RS)

The Resilience Scale (RS) was used to assess ‘resilience’, a positive personality characteristic that increases one’s ability to adapt to stress and/or adversity (Wagnild & Young, 1993). The five characteristics of resilience are: i) perseverance, ii) equanimity, iii) meaningfulness, iv) self-reliance, and v) existential aloneness (Wagnild, 2009; Wagnild & Young, 1993). The RS is a 25-item questionnaire where each item is rated on a 7-point scale and higher scores indicate greater resilience. Specifically, scores of 145 or more indicate moderately high to high resilience while scores of 120 or below indicate low resilience (Wagnild, 2009). Scores on the RS are positively correlated with life satisfaction and negatively correlated with measures of depression and health (Wagnild & Young, 1993). The test-retest reliability across the perinatal period is robust indicating that resilience, as measured by the RS, is a stable trait (Wagnild & Young, 1993). The RS has been used in individuals from varied socioeconomic backgrounds and of many different ages, including mothers and immigrants, although the need for research on the racial/ethnic differences in resilience has been identified (Wagnild, 2009). The RS was administered at visit #3 only.

Assessment of Health Postpartum – Mother and Infant

Semi-structured interviews were used to gather information about maternal and infant characteristics including: health since birth, medication use, sleeping and eating habits (including breastfeeding practices and the introduction of solid foods). To assess maternal mood in the postpartum period open-ended, general questions were used: “How has your mood been since giving birth? Did you notice any changes in your mood after giving birth and/or in the weeks/months following?”. This questionnaire was administered at visit #3 only.
2.6 Biological Tests and Sampling Protocols

2.6.1 Saliva Sampling Protocol – Mother and Infant

At visits #1, #2, and #3 participants were given a salivary cortisol sampling kit to take home and asked to complete the protocol within two weeks of the interview. Sampling kits contained sterile tubes, sample labels, written instructions and a diary (both in their language of choice). Instructions regarding the method and timing of saliva sampling were also explained verbally during the initial interview and participants were encouraged to ask questions or seek clarification at that time. The saliva sampling protocol instructed participants to provide passive drool samples at six times per day for two consecutive days: i) immediately post-waking, ii) +30-minutes post-waking, iii) +60-minutes post-waking, iv) at 12:00-1:00pm, v) at 4:00-5:00pm, and vi) at 9:00-10:00pm. This schedule is a slightly modified version of the sampling schedule proposed by Harville et al. (2007) which allows for a complete picture of both the cortisol awakening response and the diurnal profile while minimizing burden on participants and increasing compliance, and simultaneously increasing the chance of finding any potential stress-related differences (Miller et al., 2007). Collection of saliva across a minimum of two days has been recommended to account for intra-individual variations in cortisol between days (Zijlmans et al., 2015a).

The protocol further instructed participants to refrain from brushing their teeth, eating, or drinking prior to the first three samples and to refrain from eating 1 hour before the last three samples. For all samples, participants were asked to rinse their mouth with water before providing the saliva sample and to store samples in their home freezer. The diary was to be filled out on the days of saliva sampling with the date, number of hours slept at night, time of waking, and the time of day each sample was taken. Samples were brought to the clinic on ice by participants or the study coordinator/CBR and immediately placed at -20°C until analysis.
Infant salivary sampling kits, containing sterile salivettes, sterile cryovials, sample labels, written instructions and a diary (in their language of choice) were given to participants at the final study visit. Participants were instructed to obtain infant saliva samples at the same times of day as maternal samples. Samples were obtained by placing a sorbette in the infant’s mouth for 30 seconds and then placing the sorbette in the cryovial. This process was repeated for a total of two salivettes per cryovial/time point. Cryovials were then to be placed immediately in participants’ home freezer. Participants were asked to record in the infant diary, for each sample: the time of day, whether the infant was awake or sleeping and the time of the infant’s last feeding. Samples were brought to the clinic on ice by participants or the study coordinator/CBR and immediately placed at -20°C until analysis for cortisol (described in detail in section 2.7.1 below).

2.6.2 Blood Sampling Protocol – Mother

Participants underwent venipuncture by a registered nurse at visit #2 (i.e., at 32-40 weeks gestation) to yield blood samples for clinical and research purposes. Clinical measurements, assayed by the hospital laboratory, included iron (ferritin) levels, a complete blood count, and the following thyroid hormones: free T3, free T4 (thyroxine), thyroid peroxide antibodies, and thyroid stimulating hormone (TSH). An extra tube of nonheparinized (i.e., whole) blood was retained by the study coordinator for research purposes. The research-related blood samples were allowed to sit at room temperature for one hour (to allow for clot formation), and then brought to the research laboratory where they were centrifuged at room temperature for 15 minutes at 3000rpm. Serum was then drawn by pipette from the top of the blood sample, transferred to cryovials in aliquots of 0.5mL and stored in a laboratory freezer at -80°C until analysis. Serum samples were assayed by the study coordinator for serum total IgE levels and cytokines (detailed in sections 2.7.2 and 2.7.3 below).
2.6.3 Skin-Prick Testing Protocol – Mother and Infant

Skin-prick testing (SPT) with commercial allergens is a simple and relatively inexpensive method for identifying allergic sensitization with good positive and negative predictive values (Morris, 2006). There is no lower age limit for performing SPT; sensitization to both indoor and outdoor aeroallergens begins at an early age and can be detected via skin-prick testing prior to the age of 2 (Dean et al., 2007; Sheehan et al., 2010). Skin-prick testing in infants is linked with infantile eczema (Tariq et al., 2000) and is highly predictive of early sensitivity (Dean et al., 2007) and subsequent development of allergic disease (Codispoti et al., 2010; Chan-Yeung et al., 2008).

Skin-prick testing for 19 common allergens was conducted on the mother at visit #1 (i.e., early to mid-pregnancy) and on the infant at visit #3 (i.e., at > 1 year of age) by a respiratory therapist. More specifically, the SPT consisted of a positive control (histamine), a negative control (glycerin) and the following 19 allergens: fungus (2 different species), mould, dog hair, cat pelt, horse hair, feather mix, cockroach, dust mites (2 different species), cow’s milk, egg white, wheat, peanut, nut mix (no peanut), tree pollen mix, grass pollen, ragweed pollen, and weed mix (no ragweed) (ALK-Abello Inc. Pharmaceuticals; Mississauga, ON, Canada). Concentrations for each allergen extract are listed in Appendix D. The negative control consisted of NaCl 0.9%, glycerin 50%, phenol 0.4%, and deionized water. Allergen extracts were stored at 4°C in laboratory refrigerators when not in use.

The SPT procedures for mother and infant were similar but not exactly the same; specifically, they differed in the location of SPT (for mothers the SPT was administered on the forearm but for infants it was done on the back) and the type of lancet used. For maternal SPT, the participant was seated with her arm placed in a supine position on a flat surface then wiped with 70% isopropyl alcohol and allowed to air dry. The 19 common allergens plus buffer and histamine were then placed on the forearm, not using the area one inch above the wrist and 1 inch below the elbow. A pen mark was placed under each antigen for identification. For infant SPT, the mothers removed the infants’ shirt and then
held the infant in a face-to-face embrace while seated. The infants’ back was wiped with 70% isopropyl alcohol and allowed to air dry. The 19 common allergens plus buffer and histamine were then placed on the back, not using the area one inch above the buttocks and 1 inch below the shoulder. A pen mark was placed under each antigen for identification.

The allergens were applied as follows: a light prick was made by gently lifting a layer of skin with a lancet that had been dipped in a solution of allergen extract (an individual lancet was used for each allergen). For the maternal SPT each prick was done individually, resulting in a sequence of 21 pricks whereas for the infant SPT a special device was used so that all 21 pricks were done at once (i.e., 21 individual lancets were joined on one device). This resulted in a maternal SPT lasting 30-60 seconds but an infant SPT lasting only 2-3 seconds, minimizing stress for all participating parties.

Once all allergen sites were pricked a piece of tissue was gently placed over the entire area to absorb any excess antigen, being careful not to rub or smear the antigens. Participants were asked to refrain from scratching the allergen SPT site so as not to elicit a false positive reaction by increasing blood flow to the area or rubbing antigens together during the subsequent 10 minute waiting period (this is the reason the infant SPT was done on the back rather than forearm). After 10 minutes the respiratory therapist read the antigen/antibody reaction and recorded the weal and flare size of any reactions. Any questionable reactions were repeated.

2.7 Biological Assays

2.7.1 Salivary Cortisol Assay

Samples were thawed over-night and then, when thawed, vortexed for 5-10 seconds and centrifuged at 3000rpm for 15 minutes at room temperature. Samples were analyzed for cortisol levels using a commercially available enzyme immunoassay (EIA) kit according to
manufacturer instructions (Salimetrics, PA, USA). All 12 saliva samples from a single participant were analyzed in the same assay, whenever possible, to minimize variability.

The EIA test involves the use of a microtitre plate coated with monoclonal antibodies to cortisol so that cortisol in the standards and unknowns competes with cortisol linked to horseradish peroxidase for antibody binding sites. After incubation, unbound proteins are washed away and a substrate (tetramethylbenzidine, TMB) is added. The peroxidase enzyme that is bound to cortisol reacts with this substrate, producing a blue colour. The reaction is then stopped with sulfuric acid, resulting in the formation of a yellow colour. The intensity of the yellow colour therefore reflects the amount of cortisol bound to peroxidase which is inversely proportional to the amount of cortisol in the sample. The color intensity (i.e., optical density) is read on a standard plate reader (Multiskan Ascent, Thermo Scientific) at 450nm.

25 µL of controls (one high and one low), standards (6 in total, ranging from 0.33 to 82.77 nmol/L), and unknowns were pipetted, in duplicate, into appropriate wells of a 96 well microtitre plate. In addition, 25 µL of assay diluent was pipetted into i) 2 wells to serve as the zero value and ii) 2 wells with no antibody coating to serve as the nonspecific binding value. A 1:1600 dilution of the conjugate was made by adding 15 µL of conjugate to 24 mL of assay diluent and then mixing the solution. 200 µL of the diluted conjugate solution was then added to each well using a multichannel pipette and the plate was then mixed on a rotator plate at 500 rpm for 5 minutes, to ensure proper mixing, and then incubated at room temperature for 55 minutes. The incubation mixture was then removed from the plate by flicking well contents into a waste container and the wells were then manually washed four times with a solution (10 X phosphate buffered solution containing detergents and non-mercury preservative). After each wash the plate was flipped to remove the solution and then blotted on paper towels to remove any residual droplets. 200 µL of TMB solution was added to each well with a multichannel pipette, the plate was mixed on a rotator plate at 500 rpm for 5 minutes and then incubated in the dark at room temperature.
for 25 minutes. 50 µL of stop solution (3M sulfuric acid) was added to each well with a multichannel pipette to stop the reaction and the plate was then mixed on a plate rotator at 500 rpm for 3 minutes. The plate was then read at 450nm with a microtitre well reader, using Ascent v2.6 computer software. The optical density of the samples was interpolated from the standard curve to indicate the concentration of cortisol. A coefficient of variation (CV) was calculated with the software as a measure of error between the duplicates of each sample. The intra-assay CV for cortisol was 3.35% for samples with low concentrations and 3.65% for those with high concentrations. The inter-assay CV was 5.08% across ten individual runs.

2.7.2 Serum IgE Assay (for maternal blood samples)

Serum samples were thawed overnight and then, when thawed, vortexed for 5-10 seconds and centrifuged at 3000rpm for 15 minutes at room temperature. Serum IgE levels were analyzed using commercially available total human IgE enzyme linked immunoassay (ELISA) kits (Genway Biotech Inc.; San Diego, CA, USA). This assay is based on the sandwich principle, summarized below. Samples were added to plates consisting of 96 polystyrene microtitre wells coated with anti-IgE antibodies and then incubated with buffer (this allows the IgE in the samples to bind to the anti-IgE antibodies). Unbound proteins were then washed away and goat anti-IgE antibodies conjugated with horseradish peroxidase (HRP, the enzyme conjugate reagent) were added to the wells and incubated at room temperature to form complexes with the previously bound IgE, thereby creating a ‘sandwich’. Wells were washed again to remove any unbound proteins and then 3,3′,5,5′-tetramethylbenzidine (TMB), a chromogenic substrate, was added and allowed to incubate (and form a blue colour). The colour reaction was stopped with diluted hydrochloric acid (resulting in a yellow colour) and the intensity of the colour was measured by reading the absorbance at 450 nm with a plate reader (Multiskan Ascent, Thermo Scientific). This shows the concentration of IgE in the original sample because the amount of bound enzyme (represented by the colour intensity) varies in direct proportion with the concentration of IgE in the sample. Values of IgE were interpolated from the standard curve (determined
from the known standard concentrations provided in the kit) and corrected for sample dilution.

20 µL of standards (0, 10, 50, 100, 400, and 800 IU/mL of IgE in bovine serum with preservative), samples, and controls were pipetted into the appropriate wells, in duplicate. Next 100 µL of buffer was pipetted into the wells with a multichannel pipette and the shaken on a rotator plate at 500 rpm for 10 seconds, to ensure proper mixing, and then incubated at room temperature for 30 minutes. The incubation mixture was removed from the plate by flicking well contents into a waste container and the wells were then manually washed five times with a solution (10 X phosphate buffered solution containing detergents and non-mercury preservative). After each wash the plate was flicked to remove the solution and then blotted on paper towels to remove any residual droplets. 150 µL of the enzyme conjugate reagent was dispensed into each well with a microchannel pipette and the plate was shaken at 500 rpm on a rotator plate for 10 seconds before being incubated at room temperature for 30 minutes.

The incubation mixture was removed from the plate by flicking well contents into a waste container and the wells were then manually washed five times with a solution (10 X phosphate buffered solution containing detergents and non-mercury preservative). After each wash the plate was flicked to remove the solution and then blotted on paper towels to remove any residual droplets. 100 µL of TMB substrate was dispensed into each well with a microchannel pipette and the plate was shaken on a rotator plate at 500 rpm for 5 seconds before being incubated in the dark, at room temperature, for 20 minutes. The reaction was then stopped by adding 100 µL of stop solution (1N HCl) to each well with a multichannel pipette and then mixing the plate for 30 seconds at 500 rpm on a rotator plate. The plate was then read at 450nm with a microtiter well reader, using Ascent v2.6 computer software. The optical density of the samples was interpolated from the standard curve to indicate the concentration of IgE. A coefficient of variation (CV) was calculated
with the software as a measure of error between the duplicates of each sample. The intra-assay CV for IgE was 4% and the inter-assay CV was 5.5%.

### 2.7.3 Serum Cytokines Assay

Samples were thawed overnight at -20°C and then brought to room temperature, along with all assay reagents, on the morning of analysis. Samples were vortexed for 5-10 seconds and centrifuged at 3000rpm for 15 minutes at room temperature prior to analysis. Serum cytokine levels were analyzed using a commercially available human cytokine multiplex assay (EMD Millipore, Merck KGaA; Germany). More specifically, a 7-plex magnetic bead panel was used to simultaneously detect serum levels of IL-1β, IL-4, IL-5, IL-6, IL-10, TNF-α, and IFN-γ. This assay is based on the Luminex® xMAP technology, which conducts immunoassays on the surface of fluorescent-coded magnetic beads. The beads are internally colour-coded with two fluorescent dyes and it is the precise concentration of these dyes which creates a distinctly coloured bead ‘set’ that is coated with a specific capture antibody. Once the analyte from the test sample is captured by the bead, a biotinylated detection antibody is introduced and the mixture is then incubated with a conjugate (Streptavidin PE), which serves as the reporter molecule and finalizes the reaction on the surface of the bead. The beads are then passed through a laser to excite the internal dyes that mark each bead set. A second laser excites the conjugate (i.e., the fluorescent dye on the reporter molecule). Then high-speed digital-signal processors identify each individual bead and quantify the result based on fluorescent reporter signals.

To prepare the antibody-immobilized beads, each individual vial of antibody beads was sonicated for 30 seconds and vortexed for 1 minute. 60 µL of each antibody bead solution was added to a mixing bottle and 2.58 mL of bead diluent was then added to the mixing bottle to reach a final volume of 3 mL. This bead mixture was then vortexed for 1 minute. Two quality controls were reconstituted with 250 µL of deionized water, inverted several times, and vortexed for 1 minute.
times and vortexed to ensure proper mixing. These vials were allowed to sit for 5-10 minutes and then transferred to labeled polypropylene microfuge tubes.

Wash buffer was prepared by mixing the 10X wash buffer and then adding 30 mL of 10X wash buffer with 270 mL deionized water. Serum matrix was prepared by adding 1 mL deionized water to the bottle containing lyophilized serum matrix, mixing the solution well and allowing it to sit for 10 minutes for complete reconstitution. Human cytokine standards were reconstituted by adding 250 µL of deionized water, inverting the mixture several times, vortexing the vial for 10 seconds and then allowing the vial to sit for 5-10 minutes before transferring the solution to labeled polypropylene microfuge tubes. This was done for all analytes to yield a 10,000 pg/mL concentration of standard for each analyte. Working standards of 2,000, 400, 80, 16, and 3.2 pg/mL were then prepared by serial dilutions (e.g., 50 µL of the 10,000 pg/mL reconstituted standard was added to 200 µL of assay buffer and mixed well to yield the 2,000 pg/mL concentration). Assay buffer served as the 0 pg/mL standard.

200 µL of assay buffer were added to each well and the plate was then shaken for 10 minutes. The plate was then vacuumed, to remove the buffer and blotted with a paper towel. 25 µL of each standard or control was added to the appropriate wells. Additionally, for sample wells and the 0 pg/mL standard, 25 µL of assay buffer was added. Next 25 µL of serum matrix solution was added to the standard and control wells while 25 µL of sample was added to sample wells. The bottle of mixed beads was vortexed and then 25 µL of the bead solution was added to each well. The plate was then sealed and covered for incubation with agitation on a plate shaker overnight. The fluid was then removed by vacuum and each well was washed 2 times with 200 µL/well of wash buffer. Wash buffer was removed by vacuum filtration between each wash followed by gently blotting of the plate on paper towel.
Next 25 µL of detection antibodies were added to each well and the plate was sealed, covered, and incubated with agitation for 1 hour at room temperature. Then 25 µL of Streptavidin-Phycoerythrin was added to each well and the plate was sealed, covered and incubated with agitation for 30 minutes at room temperature. The contents were then removed by vacuum, the plate was washed 2 times with 200 µL of wash buffer (again, removing wash buffer by vacuum filtration between each wash followed by gently blotting of the plate on paper towel). Next, 150 µL of sheath fluid was added to each well and the plate was shaken for 5 minutes before running on the Luminex200 HTS with xPONENT software by Luminex Corporation (Austin, TX). The median fluorescent intensity was analyzed using a weighted 5-parameter logistic or spline curve-fitting method for calculating cytokine concentrations in samples. The inter-assay CV was 6.7-18.3% and the intra-assay CV was 1.6-2.9% depending on the cytokine. The minimum detectable concentration, and inter-assay and intra-assay CVs for each cytokine are reported in Appendix E.

2.8 Statistical Analyses

Data analyses were conducted using SPSS version 21. Missing demographic and questionnaire-related data due to participant-imposed time limitations or non-response were left as missing data. The amount of missing data varied according to the variable and visit. For visits #1 and #2, most variables were missing between 0-4% and no variable was missing more than 12% (see Appendix F: Table F1 for Visit #1 and Table F2 for Visit #2). For visit #3, however, 6 participants asked to complete questionnaires at home but then did not return them and/or could not be contacted for follow-up. This resulted in higher rates of missing data for visit #3 questionnaires; specifically, variables were missing 0-18% with one additional variable (RS scores) missing 29% (see Table F3 in Appendix F). Demographic and psychosocial factors were compared between groups of participants who did and did not provide saliva and serum samples using χ² analyses and t-tests.
The current study was considered a pilot study so no *a priori* effect sizes were available. The overall goal of this work was to test the feasibility of this kind of research within this population (i.e., the feasibility of this protocol). Previous research studies exploring similar themes and testing similar hypotheses have not done so using a comparable sample (i.e., a multi-ethnic community-based group of Canadian immigrant women). The uniqueness of the current study's population precluded highly specific hypotheses and all analyses were therefore considered exploratory and hypothesis generating. As such, there was more concern for Type II error than Type I error and results were therefore not corrected for multiple comparisons. In complex research designs with multiple outcomes specified *a priori* (where these outcomes are not expected to be correlated) no correction for multiplicity may be considered acceptable (Streiner, 2015). While \( p \leq 0.05 \) was considered significant here, as is the convention, all \( p \) values between 0.05 and 0.10 are also reported. Due to the pilot nature of the present study, these \( p \) values (denoting statistical trends) were considered deserving of mention as they potentially warrant further study. Inflated alpha levels are commonly used in pilot studies to shed light on potential associations that would be missed if more restrictive alpha levels were utilized (i.e., Type II error) and, therefore, be unlikely to be pursued in future research studies (McHugh, 2008). This is done with the knowledge that Type I errors will be identified as such through future, larger replication studies (McHugh, 2008).

Descriptive statistics were conducted for all variables of interest including i) range, mean, standard deviation, skewness and kurtosis for continuous measures, and ii) range, frequencies, and percentiles for nominal measures.

**Aim #1 – Is Maternal Prenatal Stress Associated with Susceptibility to Allergic Disease in Offspring?**

It was not assumed *a priori* which measure of prenatal stress would better predict allergic disease in infants and therefore one measure of life stress (CRISYS-R-neg) and one measure
of perceived stress (PSS) were analyzed as possible predictors in separate analyses. Similarly, it was not known at which time point during pregnancy prenatal stress might exert a stronger influence on infant atopic outcomes and therefore separate analyses were conducted for visit #1 (i.e., early- to mid-pregnancy) and visit #2 (i.e., late pregnancy).

**Aim1.1 – Antenatal Life Stressors**

Two binary logistic regressions were used to examine the association between prenatal maternal life stress (CRISYS-R scores, continuous measures) and infant atopy (parental report of doctor’s diagnosis of allergic disease and/or prescribed allergy medication, dichotomous measures): one with CRISYS-R-neg scores at visit #1 and one with CRISYS-R-neg scores at visit #2. Similarly, two logistic regressions examined whether there was an association between CRISYS-R scores at visit #1 and visit #2 and infant atopic predisposition (i.e., sensitization, as measured by skin prick testing, dichotomous measure). *A priori* it was decided that all four analyses would control for maternal atopy, infant sex, and infant age since these are known to significantly influence rates of infant atopy.

**Aim1.2 – Prenatal Perceived Stress**

To examine whether maternal perceived stress (PSS scores, continuous measure) and infant atopy (allergic disease diagnosis and/or prescription for allergy medication, dichotomous measure), two binomial logistic regressions were conducted (one for PSS scores at visit #1 and one for PSS scores at visit #2). Two more logistic regressions were run to examine the association between prenatal perceived stress (PSS scores at visit #1 and visit #2) and infant atopic predisposition (i.e., sensitization as measured by the SPT, dichotomous measure). Again, it was decided *a priori* that all four analyses would control for maternal atopy, infant sex, and infant age since these are known to significantly influence rates of infant atopy.
Aim #2 – What Psychosocial and Immigration-Related Factors Mediate This Association?

To examine whether psychosocial and immigration-related factors mediate any of the relationships uncovered in the analyses conducted as part of Aim #1, ideally one would enter these variables into the earlier regression models. However, sample size precluded this method since the rule of thumb for regression models is 10 cases for every predictor and the above models already each included 4 predictors for sample sizes ranging from $n = 29$ to $n = 25$. Thus, as an alternative, psychosocial and immigration-related variables were tested for a possible relationship with prenatal stress and any significant associations found were further tested for a relationship with infant atopy/atopic disposition.

Specifically, correlations were used to examine possible relationships between PSS scores at visit #1 and #2 or CRISYS-R-neg scores at visit #1 and #2 and any psychosocial or immigration-related factors measured as scale variables (e.g., maternal age, time since immigration measured in months). $t$-tests compared mean PSS or CRISYS-R-neg scores between binary psychosocial or immigration-related factors (e.g., language barrier present) while ANOVAs were used for factors with more than two levels (e.g., region of origin). Any significant mediators found using the above methods were further tested using a point-biserial correlation.

Aim #3 – Possible Underlying Mechanisms

Finally, the present study aimed to examine two mechanisms implicated in the connection between maternal prenatal stress and allergy development in infants: altered HPA axis activity and altered immune responses. The consequences of changes to HPA axis or immune activity are potentially deleterious to a wide variety of health outcomes and thus investigating this aim was deemed important in its own right (i.e., regardless of whether analyses in Aim #1 found an association between prenatal stress and infant atopy).
Specifically, this study assessed possible changes to maternal prenatal HPA axis activity, infant HPA axis activity, and maternal prenatal immune activity in relation to prenatal stress and infant atopy. Additional exploratory analyses examined whether any of the mediating variables were associated with the proposed underlying mechanisms.

**Aim #3.1 Altered Maternal Prenatal HPA Axis Activity**

This section examined whether altered HPA axis activity during pregnancy was associated with maternal prenatal stress and/or allergic susceptibility in the infant. Salivary samples were provided at both visit #1 and #2 allowing for separate analyses for early/mid- and late pregnancy. Cortisol values (day 1 and 2 separately) were subject to a log transformation and inspected for outliers, defined as ± 3 standard deviations from the mean.

Cortisol values were missing for some participants due to missing saliva tubes, broken/cracked saliva tubes, or insufficient quantity of saliva provided. At visit #1 20/684 (2.9%) samples were missing with 10 participants missing one sample, 3 missing two samples, and 1 missing four samples. For visit #2 cortisol analyses, 22/372 (5.9%) samples were missing. Specifically, 9 participants were missing one sample, 2 were missing two samples, and 1 was missing three samples. One additional participant was missing six samples because sampling was done on only one day. For this participant missing cortisol values were not imputed and cortisol measures were calculated from one day rather than the mean of two days.

For maternal samples at visit #3 6/180 (3%) samples were missing with 2 participants missing one and two samples each. For infant samples 23 had insufficient quantity of saliva for cortisol assaying and an additional 5 samples were missing, resulting in a total of 28/180 (15.6%) missing cortisol values. One infant was missing cortisol values for 8/12
samples and was therefore excluded from analyses, whereas all other infants were missing fewer than six samples (3 were missing one sample, 3 were missing two samples, 2 were missing three samples, and 1 was missing five samples). Thus for infant samples, 20 (11.1%) values were imputed.

For salivary samples at each time point Little’s MCAR test (Little, 1998) was used to examine whether cortisol values were missing at random and if so, a single randomly chosen imputation using fully conditional specification (FCS) was used to fill in missing data. Any data not missing at random were left as missing.

Diaries for salivary sampling were missing for some participants who provided saliva samples (6 for visit #1, 5 or for visit #2; 3 for visit #3). For these participants, the group mean of the number of hours slept, wake time, and times of each saliva sample were used. Additionally, for infant salivary sampling diaries 4/12 of the diaries returned were incomplete regarding the timing of infant feeding with respect to saliva sampling. These were left as missing values.

Specific cortisol variables of interest were i) awakening mean increase (CAR-MI), ii) awakening area under the curve with respect to ground (CAR-AUCg), iii) diurnal area under the curve with respect to ground (diurnal AUCg), and iv) diurnal slope. The CAR-MI was calculated by subtracting the cortisol value at waking from the cortisol value at +30-minutes post-waking (de Weerth & Buitelaar, 2005; Shea et al., 2007). The CAR-AUCg and diurnal AUCg were calculated based on the formula provided by Pruessner et al. (2003). Finally, the diurnal slope was calculated as the change in cortisol over time from waking levels to night-time levels, excluding the +30-minutes post-waking value to avoid any effect of the awakening response on the diurnal slope. Specifically, log-transformed cortisol values were regressed on time since waking (hours) separately for each participant (as others have done: Cohen et al., 2006a; Saridjan et al., 2010). These cortisol variables of
interest were calculated separately for each day of sampling based on log-transformed cortisol values and, if values for each day were sufficiently correlated, mean values were used for further analyses.

Visit #1 salivary cortisol analyses

The number of hours slept prior to the day of saliva sampling, wake time, and weeks of gestation are known to significantly influence the cortisol variables of interest (Saxbe, 2008; Vreeburg et al., 2009; Entringer et al., 2011; Karlamangla et al., 2013) and were therefore used as covariates in all cortisol analyses. Pearson correlations were used to explore whether these confounders were associated with cortisol measures in the present study; however, results of these tests did not alter the decision to use them as covariates. Partial correlations examined whether PSS scores or CRISYS-R-neg scores at visit #1 were associated with the cortisol measures, controlling for covariates.

One t-test compared the four maternal prenatal cortisol measures between infants with and without infant atopic disposition (i.e., positive SPT response). Any significant associations were further tested with an ANCOVA, controlling for weeks of gestation, wake time, and number of hours slept. Another t-test compared the maternal cortisol measures between infants with and without a physician-diagnosed allergy and again, any significant associations were tested in an ANCOVA controlling for the same covariates.

Finally, to explore whether changes in maternal HPA axis activity were associated with any of the mediators identified in Aim #2, t-tests and ANCOVAs were run (controlling for weeks of gestation, wake time, and number of hours slept). Specifically, four t-tests compared differences in each cortisol variable of interest between participants originating from different regions of the world. Next, ANCOVAs were run to test the same relationships but controlling for weeks of gestation, wake time and number of hours slept. Finally, four t-
tests compared differences in the cortisol variables of interest between participants with and without financial hardship at visit #1 and four ANCOVAs tested these associations when controlling for covariates.

Visit #2 salivary cortisol analyses

Sample sizes decreased significantly for salivary cortisol samples at visit #2 which limited the statistical analyses that could be performed. As above, Pearson correlations explored whether potential confounders including number of hours slept, wake time, and weeks of gestation were associated with the cortisol variables of interest; however, regardless of the results, these three variables were used as covariates in all further cortisol analyses. Partial correlations examined whether PSS scores or CRISYS-R-neg scores at visit #2 were associated with the cortisol measures, controlling for covariates.

Analyses of differences in cortisol measures at visit #2 by region of origin were not possible due to limited sample size (e.g., n = 6-8 per region of origin group). Likewise, sample size did not permit investigation of a possible association between prenatal maternal cortisol variables at visit #2 and with infant atopy/atopic predisposition.

Aim #3.2 Altered Infant HPA Axis Activity

Specifically, this aim sought to examine whether i) maternal and infant HPA axis activity were correlated in the postpartum period, and ii) whether infant HPA axis activity was correlated with prenatal maternal HPA axis activity. All analyses controlled for wake time and number of hours slept, with the additional covariate of infant age. Partial correlations compared maternal and infant CAR-MI, CAR-AUCg, diurnal AUCg, and diurnal slope at visit #3 while controlling for covariates. Partial correlations were conducted to compare maternal cortisol measures at visit #1 with infant cortisol measures at visit #3, controlling for maternal and infant covariates.
**Aim #3.3 Maternal Immune Parameters**

**Serum Total IgE**

Correlations examined associations between prenatal maternal serum total IgE levels and prenatal stress (PSS and CRISYS-R-neg scores) at visits #1 and visit #2. Exploratory correlations and t-tests also examined a possible association between IgE levels and depressive symptoms at visits #1 and #2. Finally, t-tests and/or ANOVAs compared whether maternal IgE levels differed according to any significant mediators identified in Aim #2.

Additionally, to examine whether serum IgE was associated with time since immigration, a correlation was conducted as well as an ANOVA with time since immigration groups. Finally, t-tests compared total IgE levels between infants with and without an atopic disposition and infants with and without a diagnosed allergy.

**Serum Cytokines**

Correlations were used to examine possible confounding factors on prenatal serum cytokine levels (IL-1β, IL-4, IL-5, IL-6, IL-10, TNF-α, and IFN-γ) or on Th1:Th2 cytokine ratios (IFN-γ:IL-5, IFN-γ:IL-4 and IFN-γ:IL-10); specifically, weeks of gestation, time of day of blood sampling, and steroid medication use. Partial correlations were conducted to examine whether prenatal serum cytokine levels or Th1:Th2 cytokine ratios were associated with prenatal stress variables at visit #2 (PSS, CRISYS-R-neg). A correlation also explored whether any of the cytokine levels or ratios were correlated with EPDS scores at visit #2, controlling for the same covariates. Likewise, t-tests or ANCOVAs examined whether cytokine levels or ratios differed according to any mediating variables identified in Aim #2. Finally, t-tests compared cytokine levels and ratios between infants with and
without atopic disposition/diagnosed allergy with the caveat that small group sample sizes severely limited power to detect differences.

3 Results

3.1 Rates of Attrition and Study Participation

Seventy-eight pregnant women were recruited into the study and assessed at visit #1. One additional woman consented but was excluded from participation due to current use of opioid medication (oxycodone) following a severe motor vehicle accident. Of the total sample, 57 (78%) attended visit #2 and 34 (44%) attended visit #3 with their infants at a minimum of 12 months post-partum. Reasons for non-participation are illustrated in Figure 3.

Figure 3: Rates of Attendance and Attrition at Each Study Visit
57 women provided saliva samples in the weeks immediately following visit #1 (Figure 4), 31 provided saliva samples following visit #2 (Figure 5) and 15 provided saliva samples for themselves and their and infants in the weeks following visit #3 (Figure 6). Blood samples were provided by 50 women during visit #2; however, only 40 were analyzed for cytokine levels (Figure 7).

Figure 4: Rates of Maternal Saliva Sampling After Visit #1 and Reasons for Non-Completion
Figure 5: Rates of Maternal Saliva Sampling After Visit #2 and Reasons for Non-Completion

- Attended Visit #2
  - N=57
  - N=25 Did Not Provide Saliva Samples at Visit #2 (were “too busy” or could not be contacted)
  - N=1 Discarded Saliva Samples (stored samples at room temperature, not in freezer)

- Provided Saliva Samples at Visit #2
  - N=31

Figure 6: Rates of Maternal and Infant Saliva Sampling After Visit #3 and Reasons for Non-Completion

- Attended Visit #3
  - N=34
  - N=19 Did Not Provide Saliva Samples at Visit #3 (were “too busy” or could not be contacted)

- Provided Saliva Samples at Visit #3
  - N=15
3.2 Descriptive Statistics

Visit #1

All participants were in their 2nd trimester of pregnancy (i.e., between 13-27 weeks gestation) except for two participants who were at 10 and 11 weeks gestation (mean = 19.1 ± 4.3 weeks). Participant ranged in age from 18-44 years (mean = 30 ± 5.3 years). Weeks of gestation and maternal age were normally distributed with no outliers. Other sociodemographic factors, collected at study intake, are presented in Table 1. For easier data interpretation, the following were dichotomized for statistical analyses: education level (graduated high school or less vs. some or completed college/university) and
maternal work status (not working or going to school vs. working or going to school or both).

To classify participants by region of origin, the UN Classification of countries by major area and region of the world was utilized (available at http://esa.un.org/wpp/excel-Data/country-Classification.pdf); however, this resulted in some groups with a very small number of participants. Thus, for analyses by region of origin, the following groups were combined: i) East Asia and Southeast Asia were combined into ‘East Asia’ group (n = 8), and ii) Europe, North America, and Oceania were combined into a single group (n = 8).

Table 1: Sociodemographic Information on Study Participants

<table>
<thead>
<tr>
<th>Sociodemographic Factor</th>
<th>n</th>
<th>Valid Percent</th>
<th>n with missing data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not married</td>
<td>4</td>
<td>5%</td>
<td>0</td>
</tr>
<tr>
<td>Education level</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Did not complete high school</td>
<td>20</td>
<td>26%</td>
<td>2</td>
</tr>
<tr>
<td>Completed high school</td>
<td>10</td>
<td>13%</td>
<td></td>
</tr>
<tr>
<td>Some or completed college/university</td>
<td>46</td>
<td>61%</td>
<td></td>
</tr>
<tr>
<td>Maternal Work status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not working or going to school</td>
<td>34</td>
<td>44%</td>
<td>0</td>
</tr>
<tr>
<td>Going to school</td>
<td>27</td>
<td>35%</td>
<td></td>
</tr>
<tr>
<td>Working</td>
<td>15</td>
<td>19%</td>
<td></td>
</tr>
<tr>
<td>Both working and going to school</td>
<td>2</td>
<td>3%</td>
<td></td>
</tr>
<tr>
<td>Paternal Work status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not working or going to school</td>
<td>16</td>
<td>21%</td>
<td>3</td>
</tr>
<tr>
<td>Going to school</td>
<td>11</td>
<td>15%</td>
<td></td>
</tr>
<tr>
<td>Working</td>
<td>47</td>
<td>63%</td>
<td></td>
</tr>
<tr>
<td>Both working and going to school</td>
<td>1</td>
<td>1%</td>
<td></td>
</tr>
<tr>
<td>Primiparous</td>
<td>25</td>
<td>32%</td>
<td>0</td>
</tr>
<tr>
<td>Number of children</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>25</td>
<td>32%</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>24</td>
<td>31%</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>13</td>
<td>17%</td>
<td></td>
</tr>
<tr>
<td>3 or more</td>
<td>16</td>
<td>21%</td>
<td></td>
</tr>
<tr>
<td>Unplanned pregnancy</td>
<td>37</td>
<td>49%</td>
<td>3</td>
</tr>
<tr>
<td>Psychiatric history</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depressive disorder</td>
<td>6</td>
<td>8%</td>
<td>2</td>
</tr>
<tr>
<td>Anxiety disorder</td>
<td>2</td>
<td>3%</td>
<td></td>
</tr>
</tbody>
</table>
Participants originated from a variety of countries, most commonly from West Asia (31%), Africa (27%), and South Central Asia (14%) (see Table 2). There was wide variation in time since immigration across the sample (0.5 – 309 months) and the distribution of this measure was both right-skewed (skewness = 1.90, SE = 0.27) and kurtotic (kurtosis = 3.52, SE = 0.54). Median time since immigration was 38.5 months (interquartile range = 15 to 83). Half of the participants had immigrated to Canada about three years prior to the study assessment (50th percentile = 38.5 months). Time since immigration was also coded as an ordinal variable, as shown in Table 2. Thirty-three participants (42%) required a translator to complete study visits.

Table 2: Immigration-Related Information for Study Participants

<table>
<thead>
<tr>
<th>Immigration-Related Factor</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Language barrier present</td>
<td>33 (42)</td>
</tr>
<tr>
<td>Area/Region of origin*</td>
<td></td>
</tr>
<tr>
<td>Asia</td>
<td>43 (55)</td>
</tr>
<tr>
<td>East Asia**</td>
<td>8 (10)</td>
</tr>
<tr>
<td>West Asia**</td>
<td>24 (31)</td>
</tr>
<tr>
<td>South Central Asia**</td>
<td>11 (14)</td>
</tr>
<tr>
<td>Africa</td>
<td>21 (27)</td>
</tr>
<tr>
<td>Latin America &amp; the Caribbean</td>
<td>6 (8)</td>
</tr>
<tr>
<td>Europe, North America, Oceania</td>
<td>8 (10)</td>
</tr>
<tr>
<td>Time since immigration</td>
<td></td>
</tr>
<tr>
<td>Less than 2 years</td>
<td>27 (35)</td>
</tr>
<tr>
<td>2-5 years</td>
<td>21 (27)</td>
</tr>
<tr>
<td>5-10 years</td>
<td>20 (26)</td>
</tr>
<tr>
<td>More than 10 years</td>
<td>10 (13)</td>
</tr>
</tbody>
</table>

*As per a modified version of the UN Classification of countries by major area and region of the world, available at [http://esa.un.org/wpp/excel-Data/country-Classification.pdf](http://esa.un.org/wpp/excel-Data/country-Classification.pdf). Europe, North America and Oceania were combined since these are listed as ‘developed economies’, in contrast to all other countries which were listed as ‘developing economies’. **East Asia = China, Malaysia, Thailand, Indonesia; West Asia = Iraq, Jordan, Syria, Lebanon, Saudi Arabia, Kuwait, United Arab Emirates, Azerbaijan; South Central Asia = Pakistan, India, Bangladesh, Iran
Most participants (61/78, 78%) were free from any major medical disorders. Of the various medical disorders present in the remainder of the sample, the most common was hypothyroidism (n = 5)\(^2\). The majority of participants (55/76, 72%) were not taking any medications during pregnancy other than vitamins. Of those taking a prescribed medication during pregnancy, the most commonly used was Diclectin® (doxylamine succinate/pyridoxine hydrochloride), the only antinauseant/antiemetic indicated in Canada for the management of nausea and/or vomiting in pregnancy (see Table 3 for a list of medications used). Only two participants (3%) said they smoked cigarettes prenatally.

### Table 3: Medication Use in Study Participants at Visit #1

<table>
<thead>
<tr>
<th>Medication</th>
<th>N=21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diclectin</td>
<td>11*</td>
</tr>
<tr>
<td>Thyroid medication</td>
<td>5*</td>
</tr>
<tr>
<td>Steroid</td>
<td>5*</td>
</tr>
<tr>
<td>Other(^6)</td>
<td>3*</td>
</tr>
</tbody>
</table>

\(^7\) n=1 Diclectin and steroid; \(n=1\) Diclectin and thyroid medication; \(n=1\) Steroid and antibiotic
\(^6\) n=1 antibiotic; \(n=1\) medroxyprogesterone; \(n=1\) venlafaxine

Rates of maternal and paternal diagnosed allergy were 20% (14/69) and 25% (16/64) respectively; however, 52% (30/58) of participants endorsed allergic symptoms that caused them to suspect an allergic disease. The most frequently endorsed item contributing to the allergenic load in the home environment was the presence of carpeting in more than 50% of the homes, which was reported by 57% (40/70) of participants. Incense was burned inside the home once a month or more by 27% (17/64) and paternal smoking was reported by 15% (10/66) of participants. Rates of pet ownership and frequent use of a humidifier were 11% (8/72) and 7% (5/72) respectively.

\(^2\) Other medical disorders reported included: \(n = 2\) high blood pressure, \(n = 2\) “ear problem”, \(n = 1\) each of polycystic ovary syndrome, endometriosis, dental problems, juvenile arthritis and biliary dyskinesia, uterine fibroid, low red blood cell count and low platelets, kidney stones, gastric ulcer.
None of the participants scored above the cut-point on the HTQ that would indicate current PTSD (i.e., score ≥ 2.5), although one participant came close with a score of 2.38. All other scores were less than 1.9 and a majority of participants (50/64, 78%) scored 1.00, indicating no exposure to any previous traumatic events.

According to the PRIME-MD PHQ, one participant had a current depressive disorder and two had a current anxiety disorder. The participant with a current depressive disorder also had an EPDS score of 17, indicating a high level of depressive symptoms and a possible depressive disorder. Conversely, two other participants with very high EPDS scores (19 and 20) did not qualify for a current depressive disorder according to the PRIME-MD PHQ.

The range, mean and standard deviation for CRISYS-R-neg, PSS, EPDS and MSPSS scores at visit #1 are shown in Table 4 (columns 2 and 3). Scores on these questionnaires were normally distributed (skewness and kurtosis values did not exceed ± 2) even though visual inspection of histograms and boxplots showed a slight right skew for EPDS and CRISYS-R-neg and a slight left skew for MSPSS.

**Table 4: Stress, Depressive Symptoms and Social Support Scores at Visits #1, #2, and #3**

<table>
<thead>
<tr>
<th>Scale</th>
<th>Visit #1 Mean ± SD (Range)</th>
<th>Visit #2 Mean ± SD (Range)</th>
<th>Visit #3 Mean ± SD (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRISYS-R-neg</td>
<td>2.4 ± 2.2 (0-9)</td>
<td>2.2 ± 2.0 (0-8)</td>
<td>2.9 ± 2.4 (0-9)</td>
</tr>
<tr>
<td>PSS</td>
<td>14.2 ± 6.4 (0-27)</td>
<td>13.6 ± 5.4 (2-25)</td>
<td>13.0 ± 6.5 (3-28)</td>
</tr>
<tr>
<td>EPDS</td>
<td>6.6 ± 4.5 (0-20)</td>
<td>6.3 ± 4.7 (0-17)</td>
<td>5.5 ± 4.8 (0-17)</td>
</tr>
<tr>
<td>MSPSS</td>
<td>70.7 ± 9.6 (44-84)</td>
<td>71.8 ± 9.5 (46-84)</td>
<td>71.5 ± 9.2 (53-84)</td>
</tr>
<tr>
<td>RS</td>
<td>-</td>
<td>-</td>
<td>142.6 ± 19.0 (97-168)</td>
</tr>
</tbody>
</table>

The PSS was moderately but significantly correlated with CRISYS-R-neg \( (r = 0.31, p = 0.009) \). Scores on the MSPSS were significantly negatively correlated with scores on the PSS \( (r = -0.33, p = 0.004) \), but not with scores on the CRISYS-R-neg \( (r = -0.10, p = 0.416) \).
EPDS scores were highly correlated with PSS scores \((r = 0.61, p < 0.001)\), but not with CRISYS-R-neg scores \((r = 0.17, p = 0.153)\). EPDS scores were negatively correlated with MSPSS scores \((r = -0.33, p = 0.003)\).

Financial hardship was reported by 21/76 (28%) of participants. No association was seen between financial hardship and education level \((p = 0.182, \text{Fisher's Exact Test})\) indicating these two measures of SES represent distinct entities in this population.

Of note, 13 women (17%) scored 12 or more on the EPDS, indicating a high level of depressive symptoms. Participants with elevated EPDS scores had higher levels of perceived stress \([t(69) = 3.29, p = 0.002]\) and lower levels of perceived social support \([t(73) = 2.27, p = 0.026]\). The distributions of CRISYS-R-neg scores in participants with high and low depressive symptoms were not normal, although they were similarly shaped across groups. There was no difference in CRISYS-R-neg scores between participants with high and low EPDS scores \([t(71) = 0.32, p = 0.749]\)^3.

Sixty-eight women (87% of the total sample) underwent skin-prick testing (SPT); 9 women declined it for fear of harm to their developing fetus and one woman declined due to undergoing similar testing within the previous 12 months. One participant underwent skin-prick testing but results were not valid due to dermatographism (i.e., she displayed reactions to the negative control, the positive control and all allergens tested) leaving 67 valid SPT results. Twenty-four participants (36%) displayed a significant reaction to one or more allergen, indicating atopy. Most participants with a positive SPT result (71%, 17/24) had a positive reaction to more than one allergen. The most common allergens to

^3^t-test using log values of CRISYS-R-neg scores + 0.01 (to avoid losing values where CRISYS-R-neg was equal to 0) also found no significant difference between high and low depressive symptoms groups \([t(71) = 0.26, p = 0.799]\).
induce a significant wheal and flare reaction were Dictyoptera (cockroach, \( n = 11 \)) and D. Farinae (dust mite, \( n = 11 \)), followed by D. Pteronyssinus (dust mite, \( n = 9 \)) and cat pelt (\( n = 9 \)). The number of allergic reactions for each of the 19 allergens tested is listed in Table 5.

### Table 5: Allergens Inducing Significant Reactions in Maternal Skin-Prick Tests

<table>
<thead>
<tr>
<th>Allergen</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dictyoptera</td>
<td>11</td>
</tr>
<tr>
<td>D. Farinae</td>
<td>11</td>
</tr>
<tr>
<td>D. Pteronyssinus</td>
<td>9</td>
</tr>
<tr>
<td>Cat pelt</td>
<td>9</td>
</tr>
<tr>
<td>Grass pollen</td>
<td>5</td>
</tr>
<tr>
<td>Ragweed pollen</td>
<td>5</td>
</tr>
<tr>
<td>Tree pollen</td>
<td>3</td>
</tr>
<tr>
<td>Peanut</td>
<td>2</td>
</tr>
<tr>
<td>Nut mix (no peanut)</td>
<td>2</td>
</tr>
<tr>
<td>Weed mix (no ragweed)</td>
<td>2</td>
</tr>
<tr>
<td>Dog hair</td>
<td>1</td>
</tr>
</tbody>
</table>

*\( n = 0 \) for Alternaria, Aspergillus fumigatus, Cladosporium, horse hair, feather mix, milk (cow’s), whole egg, and wheat

Participants with a diagnosed allergic disease were not more likely to have a positive response to the SPT than participants without a diagnosed allergy (60% versus 31% respectively, \( p = 0.147 \), Fisher’s Exact Test). Alternatively, participants who endorsed allergic symptoms were significantly more likely to have a positive reaction to the SPT compared to participants who denied experiencing allergic symptoms (56% versus 11% respectively, \( p = 0.001 \), Fisher’s Exact Test). Maternal SPT responsivity did not differ by region of origin (\( p = 0.263 \), Fisher’s Exact Test) or time since immigration (\( p = 0.645 \), Fisher’s Exact Test)\(^4\).

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\(^4\) There was also no difference in maternal SPT responsivity when the analysis was restricted to groups with \( n \geq 10 \) (i.e., 0-2 years, 2-5 years, and 5-10 years since immigration) (\( p = 0.454 \)).
Visit #2

There were no significant differences in any demographic or questionnaire scores between participants who did and did not attend study visit #2. Visit #2 occurred at a mean of 35.5 ± 2.2 weeks gestation. Forty-seven participants (82%) remained in good health and reported no pregnancy-related complications. Of the remaining participants, five developed gestational diabetes, two had placenta previa, one had placenta previa and gestational diabetes, one had intrauterine growth restriction, and one had a single umbilical artery. There was no change in medication status from visit #1 for 51 participants (89%). Three out of the six participants with gestational diabetes were now taking insulin, one participant with pre-existing hypertension was now taking methyldopa, one participant was taking an antibiotic for an acute urine infection, and one participant who was previously taking Diclectin was no longer taking it.

Mean scores on the PSS, CRISYS-R-neg, EPDS, and MSPSS questionnaires in late pregnancy did not differ substantially from those reported in early- to mid-pregnancy (Table 4, columns 4 and 5). Questionnaire scores at visit #2 were significantly correlated with scores on these same questionnaires at visit #1 ($r = 0.56$, $p < 0.001$ for PSS; $r = 0.42$, $p = 0.002$ for CRISYS-R-neg; $r = 0.34$, $p = 0.012$ for EPDS; and $r = 0.71$, $p < 0.001$ for MSPSS). Financial hardship was reported by 22/55 (40%) of participants.

Nine women (16%) scored 12 or more on the EPDS, similar to the reported rate at early- to mid-pregnancy (17%). Of note, however, five of nine of participants with high EPDS scores at visit #2 did not have elevated EPDS scores at visit #1. An additional four women had high EPDS scores at visit #1 but not at visit #2, and four participants had elevated EPDS scores at both time points (five out of the 13 participants with high EPDS scores at visit #1 did not attend visit #2).
Participants with a high EPDS score at visit #2 had significantly higher perceived stress levels at visit #2 (mean = 18.3 ± 5.6) compared to those with a low EPDS score (mean = 12.7 ± 4.9) [$t(55) = 3.12, p = 0.003$]. These participants also had significantly lower levels of social support at visit #2 (mean = 62.8 ± 9.9) compared to participants with a low EPDS score (mean = 73.5 ± 8.5) [$t(54) = 3.39, p = 0.001$].

Mean maternal total serum IgE levels were 68.78 ± 144.67 IU/mL; however, IgE levels were not normally distributed (skewness = 4.72, SE = 0.33; kurtosis = 25.64, SE = 0.64) and were therefore log transformed for further analyses. Log transformed IgE levels were normally distributed (skewness = 0.21, SE = 0.33; kurtosis = 0.27, SE = 0.64) and were therefore used for statistical analyses. The levels of total IgE did not differ significantly in participants with a diagnosed allergy (mean = 73.5 ± 51.6) compared to participants with no diagnosed allergy (mean = 72.3 ± 168.9) although the $p$ value approached significance [$t(45) = 1.84, p = 0.072$]. Of note, however, total IgE levels were significantly higher in participants with a positive SPT result (mean = 149.8 ± 240.2) compared to participants without a positive SPT result (mean = 25.5 ± 35.8) [$t(45) = 4.60, p < 0.001$]. This suggests that the SPT may be more sensitive to detection of allergy/allergic disposition compared to clinical history of diagnosed allergy in this population.

**Birth Outcomes**

Birth-related information was collected from 59 participant health records. Fourteen women (24%) delivered by Caesarian section and four of these (29%) were emergency. On average, deliveries occurred at 38.7 ± 1.8 weeks of gestation with only three infants (5%) being delivered preterm (i.e., before 37 weeks gestation). Twenty-nine (46%) infants were male, 50 (88%) were of normal birth weight while five (9%) had a high birth weight (i.e., greater than 4000g), and two (4%) had low birth weight (i.e., less than 2500g). Seventeen women (29%) experienced an intrapartum complication, most commonly abnormal fetal
heart rate or meconium. Birth weight was correlated with gestational age at delivery ($r = 0.58, p < 0.001$).

Birth weight was correlated with PSS scores at visit #2 ($r = 0.32, p = 0.036$) and EPDS scores at visit #2 ($r = 0.34, p = 0.026$); however, no other questionnaires at either visit were correlated with birth weight or gestational age. Using a cut-off score of 12 or more on the EPDS, there was a trend for gestational age to be higher in those with a high EPDS score at visit #2 [$t(40) = 1.93, p = 0.060$] but not at visit #1. There were no differences in birth weight between EPDS groups at either visit.

Visit #3

Mother-infant pairs attended visit #3 between 12-30 months post-partum (mean = 17.3 ± 4.2), and overall mothers and infants were in good health. Just over half of participants (55%, 18/33) exclusively breast-fed their infants for a minimum of six months. Scores on maternal mood, stress, and social support questionnaires at visit #3 did not differ substantially from scores at visits #1 and #2 with the exception of EPDS scores which were lower than previous EPDS scores (5.5 ± 4.8 at visit #3 vs. 6.6 ± 4.5 at visit #1 and 6.3 ± 4.7 at visit #2; see Table 4, columns 6 and 7 for visit #3 questionnaire scores). Compared to visits #1 and #2, a similar proportion scored high on the EPDS scale at visit #3 (4/30, 13%).

Scores on the Resiliency Scale (RS) ranged from 97 to 168. Scores on the RS were significantly negatively correlated with visit #3 scores on the PSS ($r = -0.47, p = 0.025$), CRISYS-R-neg ($r = -0.50, p = 0.016$) and EPDS ($r = -0.52, p = 0.009$). Furthermore, RS scores were significantly and positively correlated with visit #3 MSPSS scores ($r = 0.50, p = 0.015$). However, RS scores did not differ between participants with ($n = 3$) and without ($n = 20$) an elevated EPDS score visit #3 [$t(2.13) = 0.79, p = 0.510$]. RS scores were not correlated with
time since immigration ($r = 0.14$, $p = 0.524$), and they did not differ by region of origin [$F(5, 18) = 1.87$, $p = 0.151$].

Three mothers did not provide information about diagnosed allergies in their infants. Physician-diagnosed eczema was reported in four infants and one infant was diagnosed with an allergy to medication (amoxicillin). Thus, 5/31 infants (16% of the sample at visit #3) were diagnosed with an allergic disease. Skin-prick test results were available for 32 infants (two infants reacted very negatively during testing and the SPT was interrupted as a result). Five infants (16%) had one or more ‘clear’ positive reactions on the skin-prick test and two additional infants had one or more ‘minor’ positive reactions on the skin-prick test and a history of allergic symptoms. Thus, a total of 7/32 (22%) infants were considered to have a positive reaction to the SPT and/or an atopic disposition. Most infants (4/5) who had a clear positive reaction to an allergen had one significant wheal and flare reaction; however, one infant had three significant wheal and flare reactions. The most common allergens to cause a clear reaction in infants were egg and tree ($n = 2$ each), followed by grass, cat, and feather (all $n$’s = 1). Minor positive reactions on infant SPTs were seen for each of the following allergens: Alternaria, Cladosporium, Dictyoptera, weed mix, and peanut (all $n$’s = 1). Of the five infants with a diagnosed allergy, only 2 had a positive SPT result.

3.3 Aim #1: Is Maternal Prenatal Stress Associated with Susceptibility to Allergic Disease in Offspring?

3.3.1 Antenatal Life Stressors and Allergic Susceptibility in the Infant

A logistic regression to predict allergic susceptibility of the infant (yes/no SPT result) was conducted using infant age at the time of testing, infant sex, maternal allergic susceptibility (yes/no SPT result) and CRISYS-R-neg scores at visit #1 (number of prenatal stressful life events) as predictors. Before running the model, the Box-Tidwell test was conducted for infant age and CRISYS-R-neg scores at visit #1 and results showed that both continuous
predictors met the assumption of a linear relationship between the predictor and its logit ($p = 0.197$ for infant age and $p = 0.155$ for CRISY-R neg scores). The full model was not statistically significant ($\chi^2 = 5.92, df = 4, p = 0.205$). Nagelkerke’s $R^2$ was 0.265 and prediction success was 77.4% (96% for no infant allergic susceptibility and 14% for infant allergic susceptibility). The Wald criterion revealed that only infant sex and, to a lesser extent infant age, contributed to predicting infant allergic susceptibility, while maternal SPT result and CRISYS-R-neg scores at visit #1 were not significant predictors (Table 6). Female infants were 11 times more likely to demonstrate allergic susceptibility compared to male infants, holding all other predictors constant. For every one month increase in the infant’s age, the odds ratio of allergic susceptibility increased approximately 1.3 times.

Table 6: Logistic Regression Using CRISYS-R-neg Scores at Visit #1 to Predict Infant Skin Prick Test Response

<table>
<thead>
<tr>
<th>Predictor</th>
<th>B</th>
<th>SE</th>
<th>df</th>
<th>Exp(B)</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infant sex (female)</td>
<td>2.403</td>
<td>1.304</td>
<td>1</td>
<td>11.058</td>
<td>0.065</td>
</tr>
<tr>
<td>Infant age (months)</td>
<td>0.231</td>
<td>0.143</td>
<td>1</td>
<td>1.260</td>
<td>0.106</td>
</tr>
<tr>
<td>Maternal SPT response (positive)</td>
<td>-0.792</td>
<td>1.227</td>
<td>1</td>
<td>0.453</td>
<td>0.518</td>
</tr>
<tr>
<td>CRISYS-R-neg score at visit #1</td>
<td>-0.301</td>
<td>0.300</td>
<td>1</td>
<td>0.740</td>
<td>0.316</td>
</tr>
</tbody>
</table>

These analyses were rerun using the same predictors except CRISYS-R-neg scores at visit #2 replaced CRISYS-R-neg scores at visit #1, reducing the sample size from 31 to 27. The Box-Tidwell test for infant age and CRISYS-R-neg scores at visit #2 showed that both continuous predictors met the assumption of a linear relationship between the predictor and its logit ($p = 0.475$ for infant age and $p = 0.996$ for CRISYS-R-neg scores at visit #2). The full model was not significant and none of the predictors were significantly associated with infant SPT responsivity (see Appendix G, Table G1, for full results).
A logistic regression was conducted to predict allergic disease in the infant as diagnosed by a physician, using infant age at the time of testing, infant sex, maternal allergic susceptibility (yes/no SPT result) and CRISYS-R-neg scores at visit #1 as predictors. The Box-Tidwell test showed that infant age and CRISYS-R-neg scores at visit #1 met the assumption of a linear relationship between the predictor and its logit ($p = 0.881$ and $p = 0.905$, respectively). The full model was not significant ($\chi^2 = 4.96$, df = 4, $p = 0.291$) and none of the predictors significantly predicted allergy diagnosis in the infant (Table 7).

Next, a logistic regression using CRISYS-R-neg scores at visit #2, infant age, infant sex, and maternal SPT result as predictors of infant allergy diagnosis was conducted ($n = 26$). The Box-Tidwell test showed that the relationship between the continuous predictors and their logit was linear ($p = 0.958$ for infant age and $p = 0.958$ for CRISYS-R-neg scores at visit #2); however, as before, both the full model and all the predictors were not statistically significant (see Appendix G, Table G2, for full results).

**Table 7: Logistic Regression Using CRISYS-R-neg Scores at Visit #1 to Predict Physician-Diagnosed Allergy in the Infant**

<table>
<thead>
<tr>
<th>Predictor</th>
<th>B</th>
<th>SE</th>
<th>df</th>
<th>Exp(B)</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infant sex (female)</td>
<td>2.230</td>
<td>1.437</td>
<td>1</td>
<td>9.296</td>
<td>0.121</td>
</tr>
<tr>
<td>Infant age (months)</td>
<td>0.105</td>
<td>0.166</td>
<td>1</td>
<td>1.110</td>
<td>0.528</td>
</tr>
<tr>
<td>Maternal SPT response (positive)</td>
<td>-1.175</td>
<td>1.371</td>
<td>1</td>
<td>0.309</td>
<td>0.391</td>
</tr>
<tr>
<td>CRISYS-R-neg score at visit #1</td>
<td>-0.338</td>
<td>0.327</td>
<td>1</td>
<td>0.713</td>
<td>0.301</td>
</tr>
</tbody>
</table>

### 3.3.2 Prenatal Perceived Stress and Allergic Susceptibility in the Infant

A logistic regression to predict allergic susceptibility of the infant was conducted using infant age at the time of testing, infant sex, maternal allergic susceptibility (yes/no SPT result) and PSS scores (number of prenatal stressful life events) as predictors. The Box-Tidwell test for infant age and PSS scores at visit #1 showed that both continuous predictors met the assumption of a linear relationship between the predictor and its logit ($p = 0.510$ for infant age and $p = 0.829$ for PSS scores at visit #1). The full model was
statistically significant ($\chi^2 = 10.36$, df = 4, $p = 0.035$). Nagelkerke’s $R^2$ was 0.44 and prediction success was 80% (91% for no infant allergic susceptibility and 43% for infant allergic susceptibility). The Wald criterion revealed that infant sex and PSS scores at visit #1 significantly contributed to predicting infant allergic susceptibility (Table 8). Conversely, infant age and maternal allergic susceptibility were not significant predictors of infant SPT response. Female infants were 14 times more likely to demonstrate allergic susceptibility compared to male infants, holding all other predictors constant. For every one point decrease in PSS score at visit #1, the infant was 0.79 times less likely to have a positive SPT response.

Table 8: Logistic Regression using PSS Scores at Visit #1 to Predict Infant Skin Prick Test Response

<table>
<thead>
<tr>
<th>Predictor</th>
<th>B</th>
<th>SE</th>
<th>Df</th>
<th>Exp(B)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infant sex (female)</td>
<td>2.649</td>
<td>1.344</td>
<td>1</td>
<td>14.136</td>
<td>0.049</td>
</tr>
<tr>
<td>Infant age (months)</td>
<td>0.253</td>
<td>0.169</td>
<td>1</td>
<td>1.288</td>
<td>0.134</td>
</tr>
<tr>
<td>Maternal SPT response (positive)</td>
<td>-0.240</td>
<td>1.354</td>
<td>1</td>
<td>0.787</td>
<td>0.860</td>
</tr>
<tr>
<td>PSS score at visit #1</td>
<td>-0.238</td>
<td>0.121</td>
<td>1</td>
<td>0.788</td>
<td>0.049</td>
</tr>
</tbody>
</table>

These analyses were rerun using the same predictors except that PSS scores at visit #2 replaced PSS scores at visit #1, reducing the sample size from to 30 to 28. The Box-Tidwell test for infant age and PSS scores at visit #2 showed that both predictors met the assumption of a linear relationship between the predictor and its logit ($p = 0.777$ and $p = 0.389$, respectively). The full model was not significant ($\chi^2 = 4.35$, df = 4, $p = 0.361$) and none of the predictors were significantly associated with infant SPT responsivity (see Appendix G, Table G3 for full results).

Logistic regression to predict allergic disease in the infant as diagnosed by a physician was run using infant age at the time of testing, infant sex, maternal allergic susceptibility
(yes/no SPT result) and PSS scores at visit #1 as predictors. The Box-Tidwell test showed that infant age and PSS scores at visit #1 met the assumption of a linear relationship between the predictor and its logit ($p = 0.512$ and $p = 0.223$, respectively). The full model was not statistically significant ($\chi^2 = 2.46$, df = 4, $p = 0.651$) and none of the predictors significantly predicted allergy diagnosis in the infant (Table 9).

### Table 9: Logistic Regression Using PSS Scores at Visit #1 to Predict Physician-Diagnosed Allergy in the Infant

<table>
<thead>
<tr>
<th>Predictor</th>
<th>B</th>
<th>SE</th>
<th>df</th>
<th>Exp(B)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infant sex (female)</td>
<td>1.901</td>
<td>1.467</td>
<td>1</td>
<td>6.690</td>
<td>0.195</td>
</tr>
<tr>
<td>Infant age (months)</td>
<td>0.116</td>
<td>0.159</td>
<td>1</td>
<td>1.123</td>
<td>0.465</td>
</tr>
<tr>
<td>Maternal SPT response (positive)</td>
<td>-0.775</td>
<td>1.356</td>
<td>1</td>
<td>0.461</td>
<td>0.568</td>
</tr>
<tr>
<td>PSS score at visit #1</td>
<td>-0.007</td>
<td>0.092</td>
<td>1</td>
<td>0.993</td>
<td>0.941</td>
</tr>
</tbody>
</table>

Another logistic regression was conducted to predict infant allergy diagnosis, this time using PSS scores at visit #2, infant age, infant sex, and maternal SPT response ($n = 27$). The Box-Tidwell test found the continuous variables met the assumption of a linear relationship with their log odds ($p = 0.519$ for infant age and $p = 0.365$ for PSS scores at visit #2); however, the full model was not significant and none of the predictors significantly predicted the outcome (see Appendix G, Table G4 for full results).

### 3.4 Aim #2a) – What Psychosocial and Immigration-Related Factors Mediate This Association?

Results of the logistic regression showed that only perceived stress in early- to mid-pregnancy (i.e., PSS scores at visit #1) were associated with infant SPT responsivity. Perceived stress in late pregnancy, and stressful life events at either time, were not associated with SPT responsivity. Furthermore, none of the stress measures were associated with a diagnosis of an allergic disease in the infant. As a result, only mediators
of the association between PSS scores at visit #1 and infant SPT response were investigated.

3.4.1 Psychosocial Factors

PSS scores at visits #1 were not correlated with maternal age \((r = 0.08, p = 0.480)\). There were no differences in PSS scores at visit #1 between nulliparous and parous participants \([t(72) = 1.01, p = 0.314]\), or between participants who did and did not plan their current pregnancy \([t(69) = 0.24, p = 0.809]\). There was no difference between participants working/going to school \((n = 44)\) and those not working/not going to school \((n = 34)\) in PSS scores at visit #1 \([t(72) = 0.82, p = 0.416]\).

Two measures of SES were examined: education level and financial hardship. PSS at visit #1 was not different between high and low education groups \([t(70) = 0.33, p = 0.743]\). Scores on the PSS were not significantly different between financial hardship groups, although a trend was observed \([t(70) = 1.86, p = 0.068]\). Participants who reported experiencing financial hardship at visit #1 \((n = 19)\) had higher levels of perceived stress at visit #1 \((mean = 16.5 \pm 6.9)\) compared to participants who did not report financial hardship at visit #1 \((n = 53; mean = 13.4 \pm 6.1)\).

To investigate whether financial hardship reported at visit #1 was also associated with the outcome of interest (i.e., infant SPT response), \(\chi^2\) analyses were conducted which were significant \((p = 0.033, \text{ Fisher’s Exact Test})\), indicating that infants with a positive SPT response were more likely to be born to mothers experiencing no financial hardship in early- to mid-pregnancy. Thus, participants who did not report an experience of financial hardship in early- to mid-pregnancy had lower levels of perceived stress at that time and were subsequently more likely to have an infant with a positive reaction to the SPT. This
suggests that financial hardship was a moderator of the relationship between perceived stress in early- to mid-pregnancy and allergic susceptibility in the infant.

To further investigate the possibility of financial hardship as a mediator variable, a point-biserial correlation was conducted between prenatal perceived stress at visit #1 and infant SPT responsivity ($r = -0.38, p = 0.041$) followed by the same correlation this time partialling out financial hardship ($r = -0.29, p = 0.140$). This result suggests that financial hardship mediates the association between perceived stress and allergic susceptibility in the infant.

Since PSS and EPDS scores at visit #1 were highly correlated ($r = 0.61, p < 0.001$), depressive symptoms were another possible moderator of the relationship between PSS scores at visit #1 and infant SPT responsivity. However, maternal EPDS scores at visit #1 did not differ between infants with and without a positive SPT response ($t(28) = 0.146, p = 0.885$). Earlier analyses also illustrated that participants with elevated EPDS scores at visit #1 had higher levels of perceived stress (mean = 19.3 ± 4.3) compared to participants without elevated EPDS scores at visit #1 (mean = 13.1 ± 6.3) so elevated EPDS scores at visit #1 were another potential moderator. However, the Fisher’s exact test examining the proportion of infants with a positive SPT response in participants with and without an elevated EPDS score at visit #1 was not significant ($p = 1.000$). Scores on the MSPSS were also significantly (negatively) correlated with scores on the PSS at visit #1; however, MSPSS scores at visit #1 did not differ in infants with and without a positive SPT response ($t(30) = 0.81, p = 0.424$).

### 3.4.2 Immigration-Related Factors

There was no difference in PSS scores at visit #1 comparing women with and without a language barrier ($t(72) = 0.68, p = 0.497$). The correlation between time since immigration
(months) and PSS scores at visit #1 was not significant \((r = 0.18, p = 0.131)\). Similarly, a one-way between subjects ANOVA comparing PSS scores at visit #1 between time since immigration groups (as described in section 3.2) was also not significant \([F(3, 70) = 0.52, p = 0.671]\).

PSS scores at visit #1 did not differ between region of origin groups although it approached significance \([F(5, 68) = 2.28, p = 0.057]\). Post-hoc tests showed that participants from West Asia had significantly higher PSS scores at visit #1 (mean = 17.6 ± 6.8) compared to participants from South Central Asia (mean = 11.1 ± 7.2, \(p = 0.005\)) and participants from Africa (mean = 12.5 ± 4.8, \(p = 0.009\)). Due to very small number of participants in some groups, an ANOVA was re-run with only the groups with \(n_s > 10\) [West Asia (\(n = 22\)), South Central Asia (\(n = 11\)) and Africa (\(n = 19\))]. The one-way ANOVA was significant \([F(2, 51) = 5.40, p = 0.008]\) and post-hoc comparisons using Fisher’s LSD showed that participants from West Asia had significantly higher PSS scores than participants from South Central Asia and Africa (\(p = 0.007\) and \(p = 0.011\), respectively).

To investigate whether region of origin was also associated with infant SPT responsivity, a Fisher’s exact test was conducted examining only the proportions of infants with a positive SPT response in those groups that were previously shown to differ in PSS scores at visit #1 (i.e., West Asia, South Central, and Africa). Results did not reach statistical significance (2-sided \(p = 0.178\) but the 2 x 3 contingency table showed that of the five infants with a positive SPT response, none were born to participants from West Asia while two and three were born to participants originating from South Central Asia and Africa, respectively.
3.5 Aim #2b) What mechanisms may underlie this association?

3.5.1 Prenatal Maternal HPA Axis Activity

Saliva samples were not taken at the correct times by one participant (who had a severe language barrier and very low level of education - birth record notes “functionally illiterate in her own language”) who only took 6/12 samples at the correct time. This timing, however, still allowed for calculation of the CAR-MI but not for the other cortisol measures. Five people at both visits #1 and #2 did not fill out the sampling diaries and their missing values were replaced with the group mean (of number of hours slept, wake time, and sample collection times).

Salivary Cortisol at Visit #1 (early- to mid-pregnancy)

Cortisol values at each time point and all the cortisol measures of interest (CAR-MI, CAR-AUCg, diurn-AUCg, and diurnal slope) were moderately to highly correlated (r values ranged from 0.46 to 0.74) and therefore the mean values of day 1 and day 2 of sampling were used for all statistical analyses. Other studies using a similar salivary cortisol sampling schedule have also used mean values across two days (Tollenaar et al., 2011; Pluess et al., 2010; Edwards et al., 2001). All cortisol measures were normally distributed.

Participants who did and did not provide saliva samples did not differ in any demographics or questionnaire scores (t-tests and χ² tests all p’s > 0.05) with the exception of psychiatric history (p = 0.008, Fisher’s exact test). Visual inspection of the data suggests that participants with a history of a depressive disorder were less likely to provide saliva samples at visit #1 compared to participants with no history of a depressive disorder and that participants with a history of an anxiety disorder were more likely to provide saliva samples.
Saliva sampling occurred at a mean of 20.4 ± 4.1 weeks of gestation (range = 12-28). On average, participants who provided saliva samples slept 7.9 ± 0.9 hours (range = 6-10.5) and woke at 8am (range = 05:00-10:45). Wake time on the day of sampling was negatively correlated with the cortisol awakening response mean increase (CAR-MI) \( (r = -0.36, p = 0.007) \), the cortisol awakening response area under the curve (CAR-AUCg) \( (r = -0.40, p = 0.003) \), and with diurnal AUCg \( (r = -0.47, p < 0.001) \). The number of hours slept the previous night was negatively correlated with the diurnal AUCg \( (r = -0.52, p < 0.001) \) and with the diurnal slope \( (r = -0.27, p = 0.047) \). Weeks of gestation at the time of sampling was positively correlated with the diurnal AUCg \( (r = 0.37, p = 0.006) \). Thus, weeks of gestation at the time of saliva sampling, wake time and hours slept were all used as covariates in further analyses of visit #1 salivary cortisol.

Partial correlations between stress scores at visit #1 and cortisol measures (controlling for weeks of gestation, wake time, and hours slept) were not significant with the exception of a negative correlation between CRISYS-R-neg scores at visit #1 and the CAR-MI \( (r = -0.29, p = 0.044) \). PSS scores at visit #1 were not correlated with CAR-MI, CAR-AUCg, diurnal AUCg and diurnal slope when controlling for the covariates (all \( p \) values > 0.15). Likewise, controlling for covariates CRISYS-R-neg scores at visit #1 were not correlated with CAR-AUCg, diurnal AUCg and diurnal slope (all \( p \) values ≥ 0.15).

Comparing CAR-MI, CAR-AUCg, diurnal AUCg and diurnal slope between infants with and without a positive SPT response was significant only for CAR-AUCg \( [t(23) = 2.33, p = 0.029] \). Infants with a positive reaction to the SPT were born to mothers who displayed a lower CAR-AUCg at visit #1 (mean = 49.6 ± 17.7) compared to infants without a positive SPT response (mean = 66.6 ± 13.8). Controlling for weeks of gestation, number of hours slept, and wake time there was a significant effect of infant SPT response on the CAR-AUCg \( [F(1, 20) = 4.57, p = 0.045] \). There was also a significant effect of wake time \( [F(1, 20) = 6.02, p = 0.023] \) and a trend for an interaction between number of hours slept and infant SPT responsivity \( [F(1, 17) = 4.26, p = 0.055] \) on the CAR-AUCg (Table 10). Of note, while
covariates were normally distributed in both groups (skewness and kurtosis < 3), there was also a trend for mothers of infants with a positive SPT to provide their saliva earlier in gestation (mean = 17.6 ± 5.1 weeks) than mothers of infants without a positive SPT response (mean = 21.0 ± 3.5 weeks), $t(25) = 1.80, p = 0.085$.

### Table 10: ANCOVA Comparing Cortisol Awakening Response Area Under the Curve at Visit #1 Between Participants Whose Infants Subsequently Did and Did Not Have a Positive Skin Prick Test Response

<table>
<thead>
<tr>
<th>Sum of Squares</th>
<th>df</th>
<th>$F$ value</th>
<th>$p$ value</th>
<th>Partial Eta-squared</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weeks of Gestation</strong></td>
<td>53.525</td>
<td>1</td>
<td>0.297</td>
<td>0.592</td>
</tr>
<tr>
<td><strong>Number of Hours Slept</strong></td>
<td>3.491</td>
<td>1</td>
<td>0.019</td>
<td>0.891</td>
</tr>
<tr>
<td><strong>Wake Time</strong></td>
<td>1083.572</td>
<td>1</td>
<td>6.021</td>
<td>0.023</td>
</tr>
<tr>
<td><strong>Infant SPT Response</strong></td>
<td>821.765</td>
<td>1</td>
<td>4.566</td>
<td>0.045</td>
</tr>
<tr>
<td><strong>Infant SPT Response x Weeks of Gestation</strong></td>
<td>73.985</td>
<td>1</td>
<td>0.463</td>
<td>0.505</td>
</tr>
<tr>
<td><strong>Infant SPT Response x Wake Time</strong></td>
<td>146.559</td>
<td>1</td>
<td>0.918</td>
<td>0.352</td>
</tr>
<tr>
<td><strong>Infant SPT Response x Number of Hours Slept</strong></td>
<td>680.667</td>
<td>1</td>
<td>4.262</td>
<td>0.055</td>
</tr>
<tr>
<td><strong>Error</strong></td>
<td>3599.236</td>
<td>20</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Comparing CAR-MI, CAR-AUCg, Diurnal AUCg and diurnal slope between infants with and without a physician-diagnosed allergy showed no significant difference for any of the cortisol measures, although a borderline trend was observed for CAR-MI [$t(24) = 1.68, p = 0.107$]. Infants with a physician-diagnosed allergy were born to mothers who displayed a lower CAR-MI at visit #1 (mean = 0.03 ± 0.18) compared to infants without a diagnosed allergy (mean = 0.16 ± 0.13). However, when controlling for weeks of gestation, wake time, and number of hours slept there was no longer a significant effect of infant allergic diagnosis on the CAR-MI [$F(1, 21) = 0.73, p = 0.402$] (Table 11). These analyses also found
a trend for an interaction of infant allergy and weeks of gestation on the CAR-MI \((F(1, 18) = 3.64, p = 0.073)\).

**Table 11: ANCOVA Comparing Cortisol Awakening Response Mean Increase at Visit #1 Between Participants Whose Infants Subsequently Did and Did Not Develop a Physician-Diagnosed Allergy**

<table>
<thead>
<tr>
<th></th>
<th>Sum of Squares</th>
<th>df</th>
<th>F value</th>
<th>p value</th>
<th>Partial Eta-squared</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weeks of Gestation</strong></td>
<td>0.001</td>
<td>1</td>
<td>0.065</td>
<td>0.802</td>
<td>0.003</td>
</tr>
<tr>
<td><strong>Number of Hours Slept</strong></td>
<td>0.025</td>
<td>1</td>
<td>1.355</td>
<td>0.257</td>
<td>0.061</td>
</tr>
<tr>
<td><strong>Wake Time</strong></td>
<td>0.015</td>
<td>1</td>
<td>0.829</td>
<td>0.373</td>
<td>0.038</td>
</tr>
<tr>
<td><strong>Infant Allergy</strong></td>
<td>0.014</td>
<td>1</td>
<td>0.731</td>
<td>0.402</td>
<td>0.034</td>
</tr>
<tr>
<td><strong>Infant Allergy x Weeks of Gestation</strong></td>
<td>0.060</td>
<td>1</td>
<td>3.639</td>
<td>0.073</td>
<td>0.168</td>
</tr>
<tr>
<td><strong>Infant Allergy x Wake Time</strong></td>
<td>0.015</td>
<td>1</td>
<td>0.924</td>
<td>0.349</td>
<td>0.049</td>
</tr>
<tr>
<td><strong>Infant Allergy x Number of Hours Slept</strong></td>
<td>0.017</td>
<td>1</td>
<td>1.030</td>
<td>0.324</td>
<td>0.054</td>
</tr>
<tr>
<td><strong>Error</strong></td>
<td>0.392</td>
<td>21</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

To examine possible differences in cortisol measures at visit #1 by participants’ region of origin, comparisons were planned only for regions with a sufficiently large sample size (i.e., greater than 10), namely West Asia \((n = 18)\) and Africa \((n = 17)\). The covariates were normally distributed in each group with the exception of number of hours slept which was kurtotic in the group from Africa (kurtosis = 4.07, SE = 1.09). There were no differences between groups in the number of hours slept \([t(33) = 0.01, p = 0.99]\) or wake time \([t(33) = 0.67, p = 0.506]\). There was, however, a difference in weeks of gestation wherein the group from Africa provided saliva samples later than the group from West Asia (mean = 21.2 ± 4.1 versus 18.2 ± 3.8 respectively), \(t(33) = 2.25, p = 0.032\).
Comparing CAR-MI, CAR-AUCg, diurnal AUCg and diurnal slope between participants from West Asia and Africa showed no significant difference for any of the cortisol measures, with the exception of a trend for the diurnal slope to be lower in participants originating from Africa compared to participants originating from West Asia \([t(32) = 1.90, p = 0.066]\). Controlling for wake time, number of hours slept, and wake time however, diminished this effect to a borderline trend \([F(1, 28) = 2.85, p = 0.102]\). There was a trend for an effect of hours slept on the diurnal slope \([F(1, 28) = 3.88, p = 0.059]\) and an interaction effect for region of origin and the wake time on the diurnal slope \([F(1, 25) = 3.85, p = 0.061]\) (Table 12).

### Table 12: ANCOVA Comparing Cortisol Diurnal Slope at Visit #1 Between Participants Who Immigrated From Different Regions

<table>
<thead>
<tr>
<th></th>
<th>Sum of Squares</th>
<th>df</th>
<th>F value</th>
<th>p value</th>
<th>Partial Eta-squared</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weeks of Gestation</strong></td>
<td>&lt;0.001</td>
<td>1</td>
<td>0.523</td>
<td>0.475</td>
<td>0.018</td>
</tr>
<tr>
<td><strong>Number of Hours Slept</strong></td>
<td>0.003</td>
<td>1</td>
<td>3.875</td>
<td>0.059</td>
<td>0.122</td>
</tr>
<tr>
<td><strong>Wake Time</strong></td>
<td>0.001</td>
<td>1</td>
<td>0.770</td>
<td>0.388</td>
<td>0.027</td>
</tr>
<tr>
<td><strong>Region of Origin</strong></td>
<td>0.002</td>
<td>1</td>
<td>2.851</td>
<td>0.102</td>
<td>0.092</td>
</tr>
<tr>
<td><strong>Region of Origin x Weeks of Gestation</strong></td>
<td>0.001</td>
<td>1</td>
<td>0.902</td>
<td>0.351</td>
<td>0.035</td>
</tr>
<tr>
<td><strong>Region of Origin x Wake Time</strong></td>
<td>0.003</td>
<td>1</td>
<td>3.845</td>
<td>0.061</td>
<td>0.133</td>
</tr>
<tr>
<td><strong>Region of Origin x Number of Hours Slept</strong></td>
<td>0.001</td>
<td>1</td>
<td>1.424</td>
<td>0.244</td>
<td>0.054</td>
</tr>
<tr>
<td><strong>Error</strong></td>
<td>0.020</td>
<td>28</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Finally, \(t\)-tests found a significant difference in the diurnal AUCg and diurnal slope between participants who did and did not report financial hardship at visit #1 \([t(51) = 2.69, p = 0.010]\ and \(t(51) = 2.60, p = 0.012\) respectively) but no difference in the CAR-MI or CAR-AUCg. The covariates (weeks of gestation, number of hours slept, and wake time) did not differ between participants with and without financial hardship at visit #1. Controlling for
these covariates, diurnal AUCg remained lower in participants with financial hardship at visit #1 \( [F(1, 48) = 5.07, p = 0.029] \) (Table 13). There was also an effect of weeks of gestation \( [F(1, 48) = 8.25, p = 0.006] \), wake time \( [F(1, 48) = 10.54, p = 0.002] \), and number of hours slept \( [F(1, 48) = 8.37, p = 0.006] \) on the diurnal AUCg. However, there were also a significant interaction effect of weeks of gestation and financial hardship \( [F(1, 45) = 4.87, p = 0.012] \) and wake time and financial hardship \( [F(1, 45) = 4.57, p = 0.016] \) on the diurnal AUCg. Controlling for covariates, the diurnal slope was still lower in those with financial hardship compared to those without financial hardship at visit #1 \( [F(1, 48) = 4.39, p = 0.042] \) (Table 14).

Table 13: ANCOVA Comparing Cortisol Diurnal Area Under the Curve at Visit #1 Between Participants Who Did and Did Not Experience Financial Hardship

<table>
<thead>
<tr>
<th>Sum of Squares</th>
<th>df</th>
<th>F value</th>
<th>p value</th>
<th>Partial Eta-squared</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weeks of Gestation</strong></td>
<td>91453.768</td>
<td>1</td>
<td>8.250</td>
<td>0.006</td>
</tr>
<tr>
<td><strong>Number of Hours Slept</strong></td>
<td>92734.141</td>
<td>1</td>
<td>8.365</td>
<td>0.006</td>
</tr>
<tr>
<td><strong>Wake Time</strong></td>
<td>116842.383</td>
<td>1</td>
<td>10.540</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>Financial Hardship</strong></td>
<td>56154.378</td>
<td>1</td>
<td>5.066</td>
<td>0.029</td>
</tr>
<tr>
<td><strong>Financial Hardship x Weeks of Gestation</strong></td>
<td>110258.108</td>
<td>1</td>
<td>4.869</td>
<td>0.012</td>
</tr>
<tr>
<td><strong>Financial Hardship x Wake Time</strong></td>
<td>103550.541</td>
<td>1</td>
<td>4.573</td>
<td>0.016</td>
</tr>
<tr>
<td><strong>Financial Hardship x Number of Hours Slept</strong></td>
<td>59508.768</td>
<td>1</td>
<td>2.628</td>
<td>0.083</td>
</tr>
<tr>
<td><strong>Error</strong></td>
<td>532108.212</td>
<td>48</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Salivary Cortisol at Visit #2 (late-pregnancy)

On average, saliva samples at visit #2 were provided at 36.3 ± 1.6 weeks gestation (range = 33-40). Participants reported sleeping an average of 8.0 ± 1.0 hours the night prior to collection of saliva samples (range = 5-10.5) and waking at 08:00 (range = 05:00-10:30). Cortisol measures at visit #2 were normally distributed and showed only low to moderate correlations between each other (rs ranged from 0.05 to 0.48).

Cortisol measures at visit #1 were positively and significantly correlated with the same cortisol measures at visit #2 with the exception of the diurnal slope ($r = 0.15$, $p = 0.454$). Specifically, the correlation for CAR-MI between visits was 0.43 ($p = 0.017$), 0.62 for CAR-AUCg ($p < 0.001$), and 0.47 for the diurnal AUCg ($p = 0.009$). The covariates were not correlated with the cortisol variables of interest with the exception of a significant negative correlation between the diurnal AUCg and wake time ($r = -0.65$, $p < 0.001$) and a trend for CAR-MI and weeks of gestation ($r = -0.36$, $p = 0.056$). Nevertheless, weeks of gestation, wake time and number of hours slept were used as covariates in further analyses.

| Table 14: ANCOVA Comparing Cortisol Diurnal Slope at Visit #1 Between Participants Who Did and Did Not Experience Financial Hardship |
|-------------------------------------------------|----------------|-------------|-------------|----------------|
| Weeks of Gestation                               | Sum of Squares | df | F value | p value | Partial Eta-squared |
| Number of Hours Slept                            | 0.01           | 1  | 2.342   | 0.132   | 0.047             |
| Wake Time                                        | <0.001         | 1  | 0.282   | 0.598   | 0.006             |
| Financial Hardship                               | <0.001         | 1  | 0.034   | 0.855   | 0.001             |
| Financial Hardship x Weeks of Gestation          | 0.002          | 1  | 4.368   | 0.042   | 0.084             |
| Financial Hardship x Wake Time                   | <0.001         | 1  | 0.269   | 0.766   | 0.012             |
| Financial Hardship x Number of Hours Slept       | <0.001         | 1  | 0.151   | 0.861   | 0.007             |
| Financial Hardship x Number of Hours Slept       | 0.001          | 1  | 1.039   | 0.362   | 0.044             |
| Error                                            | 0.026          | 48 |         |         |                   |
Stress scores at visit #2 (PSS and CRISYS-R-neg) were not correlated with the cortisol measures ($r$s ranged from 0.02 to 0.30, all $p$ values > 0.15).

### 3.5.2 Infant HPA Axis Activity

In maternal samples, correlations between sampling days for each cortisol variable of interest were moderately strong ($r = 0.48$ for CAR-MI, 0.38 for CAR-AUCg, 0.45 for diurnal AUCg, and 0.32 for diurnal slope) and therefore mean values were used for further analyses. This was not the case, however, for some of the correlations between sampling days in the infant cortisol variables of interest ($r = -0.17$ for CAR-MI, 0.31 for CAR-AUCg, 0.42 for diurnal AUCg, and 0.24 for diurnal slope). Examination of data from the saliva sampling diaries suggested this may have been the result of infant feeding prior to collection of some of the samples in some participants. Data were re-analyzed after excluding four participants whose infants fed prior to saliva sampling on 3/12 (25%) or more occasions. Correlations did not change substantially ($r = -0.08$ for CAR-MI, 0.34 for CAR-AUCg, 0.40 for diurnal AUCg, and 0.23 for diurnal slope) and therefore the original data ($n = 15$ infants) were used for further analyses.

For maternal samples, the number of hours slept on the night prior to saliva sampling was not significantly correlated with any of the cortisol measures, although a negative correlation with the diurnal AUCg approached statistical significance ($r = -0.51$, $p = 0.054$). Wake time was significantly negatively correlated with the CAR-MI ($r = -0.52$, $p = 0.045$) but not with any of the other cortisol measures. Thus, number of hours slept and wake time were used as covariates in statistical analyses of maternal cortisol samples from visit #3. The correlation between the infant’s age at the time of saliva sampling and the infant’s CAR AUCg was statistically significant ($r = -0.51$, $p = 0.050$); however, infant age was not correlated with any other cortisol measures. For infant samples, the number of hours slept and wake time were not correlated with any of the cortisol measures.
The correlation between maternal and infant diurnal AUCg was significant \((r = 0.71, p = 0.021)\) when controlling for maternal wake time and number of hours slept, and infant age, wake time and number of hours slept. Similarly, the maternal and infant diurnal slope trended towards significance when controlling for all covariates \((r = 0.60, p = 0.070)\). Maternal and infant CAR-MI and CAR-AUCg were not correlated when controlling for covariates \((r = 0.15, p = 0.673\) and \(r = -0.18, p = 0.628\) respectively).

Correlations between prenatal maternal cortisol variables and infant cortisol variables, controlling for maternal and infant covariates, were significant only for CAR-MI \((r = -0.72, p = 0.046)\) although there was also a trend for diurnal slope \((r = -0.62, p = 0.098)\).

### 3.5.3 Prenatal Maternal Immune Factors

**Serum Total IgE**

Maternal total IgE levels were not correlated with PSS scores at visit #1 \((r = 0.10, p = 0.468)\) or with CRISYS-R-neg scores at visit #1 \((r = -0.11, p = 0.432)\). In contrast, maternal total IgE levels were significantly correlated with EPDS scores at visit #1 \((r = 0.31, p = 0.027)\). In addition, total IgE levels were significantly higher in participants with an elevated EPDS score at visit #1 \((\text{mean} = 138.8 \pm 162.1)\) compared to participants without an elevated EPDS score at visit #1 \((\text{mean} = 56.5 \pm 142.9)\) \([t(49) = 2.43, p = 0.019]\).

Maternal total IgE levels did not differ in participants who reported financial hardship at visit #1 \((\text{mean} = 41.2 \pm 57.1)\) compared to participants who did not report financial hardship at visit #1 \((\text{mean} = 82.1 \pm 170.7)\) although there was a trend \([t(50) = 1.84, p = 0.072]\). Log serum IgE levels did not differ by region of origin \([F(5, 47) = 0.19, p = 0.967]\).
Stress scores at visit #2 were not correlated with total IgE levels including PSS scores \((r = -0.02, p = 0.866)\) and CRISYS-R-neg scores \((r = -0.17, p = 0.229)\). Total IgE levels also did not differ in participants who did and did not report three or more recent life stressors at visit #2 \([t(49) = 1.27, p = 0.209]\). EPDS scores at visit #2 also did not correlate with levels of total IgE \((r = -0.02, p = 0.916)\), and there were no differences in total IgE levels between participants with and without an elevated EPDS score at visit #2 \([t(51) = 0.46, p = 0.646]\).

Log serum IgE levels did not differ between infants with \((n = 4)\) and without \((n = 23)\) a positive SPT response \([t(25) = 0.84, p = 0.411]\) or between infants with \((n = 2)\) and without \((n = 24)\) a physician-diagnosed allergy \([t(24) = 1.54, p = 0.136]\).

Maternal IgE levels were not correlated with time since immigration \((r = 0.06, p = 0.658)\) and, similarly, when dividing participants into groups based on time since immigration there were no group differences in mean IgE levels \([F(3, 49) = 0.91, p = 0.443]\). However Levene’s test was significant \([F(3, 49) = 3.41, p = 0.025]\) indicating unequal variances among the groups so a Welch ANOVA was conducted which also showed no significant difference in IgE levels between groups \([F(3, 21.8) = 1.00, p = 0.411]\).

**Serum Cytokines**

A large number of values for IL-5 \((30/43 or 70\%)\) were below the limit of detection and therefore IL-5 (and, consequently, IFN\(\gamma\):IL-5) was not included in further cytokine analyses. Values for all other analytes were within the limit of detection, with the exception of IL-10 where two missing values were left as missing in the data set. Serum cytokine levels, other than IL-4, were highly skewed (skewness ranged from 1.7 to 6.0) and thus were subject to a log transformation to normalize the data. One outlier (greater than 3 standard deviations) for each of the following cytokines was deleted: IL-1\(\beta\), IL-6, IL-10, and TNF-\(\alpha\). Ratios for IFN-\(\gamma\):IL-4 and IFN-\(\gamma\):IL-10 were computed and inspected for outliers; one value for IFN-\(\gamma\):IL-
4 and two values for IFN$_\gamma$:IL-10 were deleted. Following transformation and data cleaning, IL-6 remained both skewed and kurtotic (skewness = 3.8, kurtosis = 19.9) and IFN-$\gamma$:IL-4 also remained kurtotic (kurtosis = 21.1). Thus, non-parametric tests (Spearman’s rank order correlation and Kruskal Wallis H test) were employed for analyses of IL-6 and IFN-$\gamma$:IL-4.

All cytokines were significantly correlated, except for IL-1 and IL-10 whose correlation approached significance ($p = 0.061$) (see Table 15). All blood samples were collected between 10:00 and 15:45 (mean = 13:00 ± 1.6). Time of day of blood sampling was significantly negatively correlated only with IL-10 ($r = -0.41$, $p = 0.026$); however, given the known circadian fluctuation of cytokine levels (Zhou et al., 2010) all further analyses were adjusted for time of day of blood sampling. Weeks of gestation at the time of blood sampling and maternal age were not correlated with any of the cytokine levels or cytokine ratios and were not used as a covariate in further statistical analyses of cytokine levels. Only two participants who provided serum samples were taking a steroid medication and these participants had levels of serum cytokines and cytokine ratios comparable to participants who were not using steroid medication (all $p$ values for t-tests > 0.05). Thus steroid medication use was not used as a covariate in analyses of cytokine levels or ratios.

Table 15: Correlations Among Serum Cytokines At Visit #2

<table>
<thead>
<tr>
<th></th>
<th>IL-1b</th>
<th>IL-4</th>
<th>IL-6#</th>
<th>IL-10</th>
<th>IFN-$\gamma$</th>
<th>TNF-$\alpha$</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1b</td>
<td>-</td>
<td>0.60**</td>
<td>0.70**</td>
<td>0.30$^|$</td>
<td>0.57**</td>
<td>0.54**</td>
</tr>
<tr>
<td>IL-4</td>
<td>-</td>
<td>-</td>
<td>0.73**</td>
<td>0.46*</td>
<td>0.97**</td>
<td>0.86**</td>
</tr>
<tr>
<td>IL-6#</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.69**</td>
<td>0.77**</td>
<td>0.75**</td>
</tr>
<tr>
<td>IL-10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.54**</td>
<td>0.58**</td>
</tr>
<tr>
<td>TNF-$\alpha$</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.86**</td>
</tr>
<tr>
<td>IFN-$\gamma$</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</tbody>
</table>

$^a$Spearman's correlation

$^{**}p<0.001$,$^*p<0.01$,$^\|$<0.10
Partial correlations showed that neither PSS nor CRISYS-R-neg scores at visit #2 were significantly correlated with IL-1β, IL-4, IL-6, IL-10, TNF-α, IFN-γ levels or with IFN-γ:IL-4 and IFN-γ:IL-10, controlling for time of day of blood sampling (Table 16). Given the low number of mother-infant dyads with data available for both prenatal serum cytokines and infant SPT responses ($n = 19$ to $21$ and $n = 2$ infants with a negative and positive SPT respectively), it was deemed prudent to conduct only exploratory analyses that did not control for time of blood sampling. $t$-tests found no difference in maternal prenatal IL-1β, IL-4, IL-10, TNF-α, IFN-γ levels and the IFNγ:IL-10 between infants with and without atopy. Mann-Whitney $U$ tests found a a trend for infants with a positive SPT response to come from participants with lower prenatal IFN-γ:IL-4 compared to infants without a positive response to SPT ($U = 4.0, p = 0.078$) but no difference in IL-6. There was insufficient sample size to examine differences in cytokine levels or ratios between infants with and without a diagnosed allergy.

<table>
<thead>
<tr>
<th></th>
<th>PSS</th>
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<th>CRISYS-R-neg</th>
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<tr>
<td></td>
<td>$r$ value</td>
<td>$p$ value</td>
<td>$r$ value</td>
<td>$p$ value</td>
</tr>
<tr>
<td>IL-1β</td>
<td>-0.15</td>
<td>0.454</td>
<td>-0.19</td>
<td>0.317</td>
</tr>
<tr>
<td>IL-4</td>
<td>0.10</td>
<td>0.593</td>
<td>0.06</td>
<td>0.753</td>
</tr>
<tr>
<td>IL-6$^*$</td>
<td>0.02</td>
<td>0.908</td>
<td>0.00</td>
<td>0.990</td>
</tr>
<tr>
<td>IL-10</td>
<td>0.18</td>
<td>0.370</td>
<td>0.30</td>
<td>0.136</td>
</tr>
<tr>
<td>TNF-α</td>
<td>-0.13</td>
<td>0.499</td>
<td>-0.01</td>
<td>0.955</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>0.11</td>
<td>0.562</td>
<td>0.05</td>
<td>0.802</td>
</tr>
<tr>
<td>IFN-γ:IL-4$^*$</td>
<td>-0.29</td>
<td>0.125</td>
<td>0.04</td>
<td>0.841</td>
</tr>
<tr>
<td>IFN-γ:IL10</td>
<td>-0.26</td>
<td>0.192</td>
<td>-0.11</td>
<td>0.599</td>
</tr>
</tbody>
</table>

$^*$Nonparametric partial correlations
A MANCOVA was not appropriate to examine differences in cytokine levels and ratios between participants who did and did not experience financial hardship in early-/mid-pregnancy, controlling for time of day of blood sampling because assumptions for this test were not met: i) the dependent variables were highly correlated ( multicollinearity), and ii) the covariate was not linearly related to all dependent variables. Therefore a partial point-biserial correlation was conducted and showed that there were no significant differences in cytokine levels or ratios between participants who did and did not experience financial hardship in early to mid-pregnancy controlling for time of day of sampling (all t-test p values > 0.10) although the correlation between IL-10 and financial hardship approached a trend (r = 0.32, p = 0.101).
4 Discussion

Results are discussed according to the study aims with significant findings examined first followed by data trends (which are considered note-worthy given that the analyses have been conducted in an understudied population); finally, non-significant results are briefly considered and placed within the context of previous research. Particular attention is given to the analyses of maternal HPA axis activity given that this is the mechanism implicated most often in programming of development and health yet, to date, the data on predictors and consequences of prenatal cortisol are largely conflicting. Furthermore, it was suggested recently that in studies of prenatal cortisol, authors should report analyses with and without confounders to allow for comparability of results (Zijlmans et al., 2015a).

Here a link between lower levels of perceived stress during early- to mid-pregnancy and subsequent allergic susceptibility in the offspring was seen, but this association was mediated by the experience of financial hardship during pregnancy. Maternal prenatal HPA axis activity demonstrated stronger associations with prenatal maternal stress, financial hardship, and infant atopy than measures of prenatal immunity. Results must be interpreted with caution due to the small sample size and multiple statistical tests employed. Nevertheless, given the novelty of sampling in an ethnically-diverse group of immigrant women, and the discoveries that emerged therein regarding facilitators and challenges to conducting research in this population, the present results make an important contribution to the literature.

4.1 Aim #1: Is Maternal Prenatal Stress Associated with Susceptibility to Allergic Disease in Offspring?

Prenatal Perceived Stress

The hypothesis that prenatal stress is associated with allergic disease susceptibility in the infant was only partially supported by the present study's results. A trend was found for
lower levels of maternal perceived stress in early- to mid-pregnancy to be associated with greater odds of atopy in infants aged 12-30 months. This association was not seen in late pregnancy. To date, no other studies have examined a link between prenatal perceived stress and infant atopy assessed using skin prick testing (SPT), although one previous study used another measure of atopy in the child and found a conflicting result (Reyes et al., 2011).

In a sample of urban, minority women of low SES, Reyes et al. (2011) measured atopy in the child at ages 2, 3, and 5 using allergen specific IgE and found no association with prenatal maternal psychological distress. The most significant differences between this and the present study are in the measures of prenatal stress and the samples. In the Reyes et al. study, psychological distress, termed ‘demoralization’, included domains such perceived physical health, poor self-esteem, confused thinking, and psychophysiological symptoms that may not be related to stress per se but rather to other psychological problems. Moreover, there was some evidence that demoralization was a stable trait since prenatal and postnatal demoralization were significantly associated. This is in contrast to perceived stress measured using the PSS which is thought to be reflective of an individual’s current state. For example, in the original publication of the PSS, Cohen et al. (1983) describe only modest correlations in the PSS when test-retesting was done 6 weeks apart. Furthermore, the Reyes et al. study sample consisted of African American and Dominican women and had a relatively large sample (and was therefore able to statistically control for multiple confounders such as maternal education, maternal asthma, and secondhand smoke exposure). In contrast, the present study had a very small sample that consisted of women from highly diverse racial, ethnic and SES backgrounds. These differences may account for the disparate findings; however, given the lack of studies measuring prenatal stress and infant/child atopy further research is clearly needed.

One other study is worth mentioning in this context. Cookson et al. (2009) used data from a large-scale population cohort (Avon Longitudinal Study of Parents and Children, ALSPAC
study) to examine the association between prenatal maternal anxiety and childhood asthma at age 7. SPTs were also conducted in the children; however, analyses only examined whether associations differed in atopic and non-atopic asthma but not whether prenatal anxiety and SPT responses were related. In other words, SPT results were used as a moderating but not an outcome variable. Considering the ALSPAC data have already been gathered and the sample size is substantial, study contributors should consider using data from this cohort to test the relationship between maternal prenatal anxiety and childhood atopy.

Unlike other cohort studies, here no association was seen between perceived stress and physician-diagnosed allergies in the infant at 12-30 months of age. Three previous studies have examined this hypothesis, although measures of prenatal distress and the specific allergic outcome measured differed across all studies. Two studies defined psychological/emotional stress during pregnancy as a composite of depression, anxiety and stress (although different scales were used between studies): one found a positive association with eczema in infants aged 2 years (Wen et al., 2011), and the other found a significant association with persistent wheezing at 1-4 years of age (Guxens et al., 2014). The second study also found a trend for an association between subjective distress in mid-pregnancy and physician-diagnosed asthma at age 6 (Guxens et al., 2014). Finally, a small-scale prospective study found an increased risk of doctor-diagnosed asthma at age 12 in girls born to mothers who reported greater prenatal stress in response to a natural disaster (Turcotte-Tremblay et al., 2014).

The lack of association in the present study between prenatal perceived stress and allergic disease in the infant may be attributed to the assessment of a relatively mild form of stress which was analyzed as a continuous score. Previous studies found relatively high levels of prenatal stress predicted allergic disorders. The Guxens et al. study reported significant associations only in participants with clinically significant psychological distress (8% of the total sample) and the Wen et al. study found the association only in participants in the top
tertile of emotional stress scores. Finally, the Turcotte-Tremblay study consisted entirely of participants who were exposed to a severe and potentially traumatic life stressor, and prenatal stress was measured as the subjective response to this rather extreme stressor. Given the present study’s limited sample size, greater statistical power was achieved by analyzing the data along a continuum. However, it is conceivable that the relationship between prenatal perceived stress and childhood allergies only emerges in cases of very high levels of distress and therefore the type of statistical tests chosen in the present study precluded detecting this association.

Prenatal Life Stressors

The absence of a link between prenatal life stressors and allergic disease/sensitization in the infant reported here contradicts results of several large-scale studies. The most compelling evidence is from a recent birth cohort study where mothers who experienced adverse events during the second half of their pregnancy were found to have higher odds of asthma and eczema in their children at age 14 (Hartwig et al., 2014). Of note, no association was seen between life stressors during pregnancy and atopy in the child as measured by skin prick test or specific IgE. The strength of these results arises from the thoroughness of both the study design and statistical analyses: a stringent definition of allergic disease was used, atopy was assessed with two parameters (skin prick test and specific IgE at ages 6 and 14), a large sample size was used, the effects of stressful life events during both halves of pregnancy were examined separately, and multiple salient confounders were used as covariates. Furthermore, this is the first and only study with a follow-up period into adolescence in a large sample.

There are some key differences between the present study and the Hartwig et al. study that could account for the discrepant findings, the most salient of which is the sample size. In particular, the present study had limited power to detect an association in late pregnancy where Hartwig et al. found an effect of prenatal adverse events. Secondly, Hartwig et al.
(2014) report that the association found at age 14 was not found at age 6 suggesting that early diagnoses of allergic disease may represent transient states rather than true allergic disorders. If this is the case, it would apply also to the present results where allergic disease outcomes were measured at an even earlier age. Finally, the association between prenatal adverse life events and allergic disease in children was stronger in cases where mothers did not have asthma. Here data were not analyzed separately based on maternal asthma status although maternal atopy was used as a covariate. Nevertheless, the present study analyzed asthmatic and non-asthmatic mothers jointly in a small sample size and this may have obscured the detection of an association between prenatal SLEs and allergic disease in the offspring. Given that Chiu et al. (2012) also found an association between prenatal SLEs and childhood wheeze up to the age of 2 only in non-atopic non-sensitized mothers, future studies should stratify analyses by maternal atopy/asthma.

Looking at allergic outcomes in younger children, two studies found prenatal stressful life events were associated with allergic disease in the first few years of life. However, as in the prenatal perceived stress literature, the association tended to emerge only under circumstances of extreme stress. For example, in a birth cohort study Sausenthaler et al. (2009) showed that prenatal maternal exposure to SLEs was associated with a higher risk of eczema in the child’s first 2 years of life; however, a cut-off score (of 2 or more SLEs) was used which represented only the top 5% of the study population. Furthermore, the SLEs were primarily physical in nature (e.g., premature labour, bleeding before 28 weeks gestation) with only a few psychosocial stressors, suggesting their findings could have been related more to biological changes from underlying medical morbidities than life stress per se. A population-based Swedish study found bereavement of a close relative during pregnancy, especially if experienced in the 2nd trimester, was associated with asthma at age 1-4 in boys but not girls (Fang et al., 2011). On the one hand, the gravity of this particular stressor and the large sample size provide strong evidence for an effect of prenatal life stress on asthma development in offspring. On the other hand, these results do not inform the question of whether milder, more common stressors may contribute to allergy/asthma development in the next generation.
Prenatal stressful life events have also been linked with increased rates of childhood allergic disease in a cross-sectional study of children aged 3 to 14 (deMarco et al., 2012). Of note, asthma, allergic rhinitis, atopic eczema, and wheezing were assessed separately and higher rates of each disease were found in children whose mothers had experienced at least one stressful life event during pregnancy. Again, stressful life events were confined to a few severe life stressors (divorce, mourning, or loss of job) rather than a wide range of psychosocial and environmental stressors such as those measured in the present study with the CRISYS-R scale.

Two prior studies have used the CRISYS-R scale to examine the effects of prenatal life stressors on infant health; however, both studies used infant wheeze as their outcome of interest (Chiu et al., 2012; Sternthal et al., 2011). These studies did find a link between SLEs in pregnancy and subsequent wheeze in children up to the age of 2 (Chiu et al., 2012; Sternthal et al., 2011); however, the relevance to allergic disease development is not entirely clear since wheezing and asthma are not synonymous. Wheezing is often used as a proxy for asthma in research studies of young children because at that age a diagnosis of asthma is virtually impossible (e.g., the Childhood Asthma Prevention Study, Almqvist et al., 2007; the Multicenter Allergy Study, Illi et al., 2006). However, wheezing is increasingly recognized as a heterogeneous phenotype which is associated with asthma development only in a fraction of cases (Morgan et al., 2005), usually in those with atopy (Illi et al., 2006). Thus a substantial portion of children with wheeze in early life appear to lose these asthmatic symptoms later in life and therefore reliance on ‘wheeze’ in infancy as an outcome measure is problematic.

Another factor that may account for the discrepancy between previous studies using the CRISYS-R scale and results of the present study is that life stressors were previously defined as the number of domains with at least one negative life stressor endorsed while
here a tally of negative life stressors was used. In exploratory analyses, the number of domains with at least one negative life stressor was very highly correlated with the total number of negative life events \((r = 0.87, p < 0.001)\) and so the latter definition was used to maintain consistency with previous studies using allergic outcomes in infants rather than wheeze-related outcomes.

**Summary**

Overall, the present results lend some, albeit minor, support to a growing body of research showing a link between maternal mental health during pregnancy and susceptibility to allergic disease. Of note, the results show an effect in the opposite direction of what has been reported in previous large-scale studies and the small size and heterogeneity of the current sample suggests caution is warranted in drawing any major conclusions from results presented herein. The relationship between low prenatal perceived stress and the infant’s SPT response is nevertheless of interest since SPT in the early years is a good predictor of future allergic disease development. A positive SPT response before the age of 2 significantly predicts subsequent allergic disease at age 5 to 6 (Ro et al., 2015; Almqvist et al., 2007) and most infants showing a positive SPT response before the age of 3 have an allergic disease by age 6 (Kjaer et al., 2009).

The lack of association between prenatal stress and physician-diagnosed allergy is likely related to the low prevalence of this outcome in this age group coupled with the limited sample size. Additionally, while physician-diagnosed allergy is a reliable measure of allergic disease, it is also reflective of access to care and may be subject to reporting bias. The finding that stressful life events during pregnancy were unrelated to subsequent allergic susceptibility in the infant may also be related to the small sample size or, alternatively, to the different measurement of SLEs used in the present study compared to previous studies.
4.2 Aim #2a) What Psychosocial and Immigration-Related Factors Mediate This Association?

4.2.1 Financial Hardship

In the current study, financial hardship mediated the association between perceived stress and subsequent infant allergic sensitization. A trend was found for higher perceived stress in participants who experienced financial hardship, a finding that is not particularly surprising given that financial hardship/low income is a well-known stressor that contributes to prenatal stress and depressive symptoms (Kane et al., 2014; Kingston et al., 2012; Abdou et al., 2010; Goyal et al., 2010). Previous research has shown this to be true in both Canadian-born and immigrant women living in Canada (Miszkurka et al., 2012). The finding that participants who experienced financial hardship in early- to mid-pregnancy were less likely to have an infant with allergic sensitization is also consistent with previous research showing that high SES is associated with a greater prevalence of allergic disease (Uphoff et al., 2015).

Overall, the literature on SES and asthma/allergies in children is confusing and complicated. Some studies have found low SES is associated with the development of asthma and rhinitis in children (Gong et al., 2014; Almqvist et al., 2005; Lindbaek et al., 2003) as well as higher prevalence rates and poor outcomes in childhood asthma (Keet et al., 2015; Kozyrskyj et al., 2010; Poyser et al., 2002; Litonjua et al., 1999). Other studies link high SES with childhood atopy (Hamid et al., 2015; Silva et al., 2010; Hancox et al., 2004) and atopic dermatitis (Fu et al., 2014; Stelmach et al., 2014; DaVeiga, 2012; Schmitz et al., 2012; Shaw et al., 2011; Gruber et al., 2002; Werner et al., 2002; Bergmann et al., 2000). Finally, still other studies have found no relationship between SES and total serum IgE in children (Hancox et al., 2004) or childhood asthma prevalence (Hancox et al., 2004; Aligne et al., 2000; Mitchell et al., 1989).
Several factors likely contribute to these discrepant results. First, the majority of studies linking low SES with childhood allergic disease outcomes focus on asthma, rather than atopy/allergic diseases; however, the aetiology and clinical course of asthma is complex and includes both atopic and non-atopic phenotypes (Sly et al., 2008; Martinez et al., 2002). A recent systematic review of the association between SES and asthma/allergy (not confined to childhood) concluded that low SES is associated with asthma while high SES is associated with allergy prevalence (including allergies in general, atopic dermatitis and allergic rhinoconjunctivitis) (Uphoff et al., 2015). Second, the type of asthma-related outcome is critical since some evidence suggests high SES is associated with a higher prevalence of asthma, while low SES is associated with greater asthma severity and morbidity (Poyser et al., 2002). Comparisons of childhood asthma rates and severity internationally support this notion as asthma prevalence is generally higher in more affluent countries; however, asthma symptoms are more severe in countries that are less affluent (Lai et al., 2009). Third, the association between allergic sensitization and allergic symptoms varies by SES such that in high SES children sensitization was linked with clinical symptoms while in low SES no association was seen (Hamid et al., 2015). Again, epidemiologic evidence appears to support this as the link between sensitization and asthma symptoms is moderated by economic development since the association is strongest in countries with high gross national income per capita (GNI) and weaker in those with lower GNI (Weinmayr et al., 2007). This has led some to suggest a link between higher SES and allergic sensitization on the one hand, and lower SES and asthma/allergic disease morbidity on the other (Silverberg & Simpson, 2014).

Other reasons for disparate findings regarding SES and childhood asthma/allergies relate to the definition of SES. Some studies use a single SES indicator (e.g., parental education or occupation) which may be differently related to other singular SES indicators (e.g., household income) in some populations (e.g., single-parents households, immigrant families). Finally, the measurement of SES as a snap-shot, rather than changes over time, may be misleading. One study found that chronic low income was linked with the
Nevertheless, results of the present study are consistent with other studies that have found a link with childhood atopy, specifically SPT responsivity, and higher SES (Hamid et al., 2015; Silva et al., 2010; Hancox et al., 2004). Higher rates of positive SPT responses have also been found in adults with higher SES (Skaaby et al., 2015; Ferraz et al., 2011). This association is presumed to be the result of higher rates of environmental risk factors for atopy in higher SES populations, specifically increased cleanliness as per the ‘hygiene hypothesis’. This influential hypothesis, formulated to account for the negative relationship between family size and hayfever (Strachan, 1989), originally posited that an environment which is ‘too clean’ leads to fewer infections which may, in turn, predispose to allergic disease. There is a large body of evidence supporting the hygiene hypothesis, specifically with respect to protective effects of a) exposure to farm animals and b) larger family size on the development of allergic disease (von Mutius, 2010). These protective effects appear to be mediated by exposure to specific microbial factors, especially during early life (including *in utero*) (Garn & Renz, 2007), although chronic exposures and exposures later in life also play a role (Brooks et al., 2013). Perinatal microbial exposure as it pertains to the development of the immune system and the subsequent propensity for allergic disease is currently an active area of research.

In the present study, it appears that the link between infant sensitization and low levels of prenatal stress was primarily driven by high SES, defined here as a lack of financial hardship during pregnancy. This is consistent with the hygiene hypothesis; however, precise measurement of environmental exposures is needed to verify and understand this link given that no previous studies have examined these associations concurrently in an immigrant population.
4.2.2 Region of Origin

Region of origin was not a significant mediator of the association between prenatal perceived stress and subsequent allergic sensitization in infancy; however, some trends in the data are worth noting. First, participants from West Asia had significantly higher levels of prenatal perceived stress than participants from South Central Asia and Africa (the latter two regions did not differ significantly from each other). This is in line with a previous Canadian longitudinal study of ethnic differences in prenatal mental health and its association with pregnancy and infant health outcomes where women who self-identified as Arab had the highest prevalence of elevated scores on the PSS in the 2\textsuperscript{nd} trimester (Robinson, 2013). In that study, nearly 40\% of pregnant Arab women scored above the 20\% percentile in PSS scores while the rate in the “Other Asian” group was about 33\%, and 29\% in the Black/African American group. Of note, while these ethnic groups included both Canadian- and foreign-born women, a majority (74-81\%) within each ethnic group were foreign-born. While the categorization of region of origin utilized in the present study differed somewhat from the ethnicity groups used in the Robinson study, there is significant overlap. In the present study, all but one participant in the West Asia group originated from a country in the Middle East, where Arabic was the native tongue and Islam the religion. Thus results here corroborate previous research suggesting greater perceived stress in women of West Asian origin and/or Arab ethnicity living in Canada.

It is unclear why participants originating from West Asia may have reported more perceived stress. One possibility is that a larger proportion of participants from West Asia experienced financial hardship (11/24, 46\%) during pregnancy compared to participants from Africa (5/19, 26\%) and South Central Asia (2/11, 18\%) and financial hardship is associated with more stress (as previously described). Additionally, financial difficulties may have been perceived as more stressful for this group of women since income level is a predictor of prenatal anxiety in some ethnic groups but not in others (Gurung et al., 2005). Research on ethnocultural differences in the experience of perinatal distress is limited, however, especially involving pregnant women of more varied cultural/ethnic backgrounds than Hispanic/Latina, ‘Black’ and ‘Asian’. Studies on ethnic differences in
postpartum depression are relatively more common, however, and may shed some light. Middle Eastern migrant women in Australia with postpartum depression (PPD) described fear of being labeled a bad mother if they were seen as not coping and/or complaining about difficulties during the perinatal period, a time when they should feel happy and positive (Nahas et al., 1999). One could speculate that the incongruence between experiencing hardships/stresses and not being able to express emotions related to them could create even greater distress, especially when compounded by a lack of social support.

In a study of women from West Africa with PPD, some women admitted they did not express their difficulties due to distrust of others and fear of stigma and gossip (Gardner et al., 2014). A Canadian study of new immigrant women from India found that family members encouraged women to be healthy and free of stress during their pregnancy and these women reported that prayer was an important element in helping them to achieve a calm state (Grewal et al., 2008). Thus, minimizing and/or under-reporting of stress may have been a factor in the low levels of perceived stress in participants from Africa while low levels of perceived stress in participants from South Central Asia may have been related to social and/or religious practices.

Differences in actual or perceived social support across different ethnic groups may also have contributed to disparate rates of perceived stress. In the study by Robinson (2013), Arab women displayed the highest prevalence of prenatal depressive symptoms and anxiety (in addition to perceived stress) and the lowest levels of perceived social support. Here, exploratory analyses illustrated a similar pattern with respect to social support. Perceived social support was significantly lower in participants from West Asia (mean = 66.0; $p = 0.015$) and marginally lower in participants from Africa (mean = 68.1; $p = 0.066$) compared to participants from South Central Asia (mean = 74.5). This is in line with at least one other study of Canadian immigrant women from India where women described extensive emotional and practical support provided by family members in the perinatal period (Grewal et al., 2008).
Mental health studies have found ethnocultural, linguistic and religious differences in the experience, description, and/or reporting of stress (Aldwin, 2004) and coping styles (Kuo, 2011). Unfortunately, the majority of research on cultural differences in coping styles has focused on Asians, African Americans, and Latino/Latina Americans with scant research on other cultural groups such as Arabic and Middle Eastern groups (Kuo, 2011). In Canada, the Middle Eastern population (including Arabs and West Asians) is the fastest growing visible minority group (Belanger & Malenfant, 2005) and therefore more research on the determinants of stress and/or depression in this population is urgently needed, especially during the perinatal period when significant consequences on both mother and child can occur.

The rate of infant sensitization in the present study differed by region of origin but this did not reach statistical significance, likely due to the limited sample size. The highest rate of allergic sensitization was in infants born to mothers from Africa \((n = 3/7)\), followed by those from South Central Asia \((n = 2/5)\) while no infants born to mothers from West Asia were sensitized \((0/6)\). In adults, markers of allergic disease (e.g., total serum IgE, allergic sensitization) vary by ethnicity with Black and Hispanic women displaying greater proportions of atopy even after adjusting for SES and environmental variables (Wegienka et al., 2012; Litonjua et al., 2005). This is in line with a recent review of racial differences in allergic sensitization across all age groups which concluded that African American/Black individuals are more often sensitized compared to White individuals and that this racial disparity emerges by the age of two years (Wegienka et al., 2013). However, little is known about how these compare to sensitization rates and patterns in other racial/ethnic groups (Wegienka et al., 2013).

Rates of sensitization in children as measured by SPT in the International Study of Asthma and Allergies in Childhood (ISAAC) Study varied widely by country (from 1.7% in rural Ghana to 45.3% in Hong Kong, China) (Weinmayr et al., 2007). As valuable as these data are, it is hampered by the fact that most countries had only one participating study centre
whose sensitization rates may not have been representative of the entire nation. Specifically, a greater prevalence of allergic sensitization and allergic disease are seen in persons living in urban versus rural areas (Schmitz et al., 2012; Shaw et al., 2011; Yang et al., 2008; Litonjua et al., 2005; Aligne et al., 2000). Air pollution likely contributes to these findings since children exposed to greater levels of traffic-related air pollutants have an increased risk of both atopic sensitization and allergic disease (Morgenstern et al., 2008). Thus, the present study and some previous studies have found differences in atopy across world regions, races, cultures and ethnicities; however, more research on this topic is needed.

**Summary**

The experience of financial hardship during pregnancy mediated the association between prenatal perceived stress and allergic sensitization in the infant but region of origin, while displaying some features of a mediating variable, did not. The former is in accordance with previous research on the role of SES as a contributor to prenatal stress and a contributor to allergic sensitization although no previous studies examined these associations concurrently in an immigrant population. The inability to detect a mediating effect of region of origin is likely related to the limited number of participants per group in the final sample; however, results are still noteworthy considering the dearth of research on this topic and the increasing proportion of the Canadian population who are immigrants (both first- and second-generation).

### 4.3 Aim #2b) What Mechanisms May Underlie This Association?

#### 4.3.1 Prenatal Maternal HPA Axis Activity

Overall, the known confounders of salivary cortisol activity showed a similar pattern in the present study as those reported previously suggesting that the findings with respect to salivary cortisol in the present study (outlined below) are likely valid. The cortisol awakening response (CAR-MI and CAR AUCg) was smaller in those who woke up later,
consistent with previous studies (Vreeburg et al., 2009; Kivlighan et al., 2008; Kudielka & Kirschbaum, 2003; Edwards et al., 2001). The diurnal AUCg was also lower in women who woke later, mirroring prior research showing lower cortisol levels throughout the day in late awakeners (Karlamangla et al., 2013; Edwards et al., 2001). Women who slept longer on the night before saliva sampling had a smaller diurnal AUCg and flatter slope, as others have found (Karlamangla et al., 2013) perhaps due to waking up later (and therefore having a blunted CAR). Alternatively, others found the diurnal slope was steeper in persons who slept longer and in those who woke up later in the morning (Kumari et al., 2009; Vreeburg et al., 2009). This discrepancy may reflect differences between studies in sample characteristics and/or participant compliance with saliva sampling protocols. The timing of saliva sampling with respect to weeks of gestation was associated with the diurnal AUCg in line with previous reports of increasing maternal cortisol levels as pregnancy advances (Kane et al., 2014; Hompes et al., 2013; Voegtline et al., 2013; Kaasen et al., 2012; Bolten et al., 2011; Davis & Sandman, 2010; Sarkar et al., 2006; Obel et al., 2005; Mastorakos & Illias, 2003).

**Associations with Prenatal Maternal Stress**

The number of stressful life events experienced in the previous 6 months was negatively correlated with the CAR mean increase (CAR-MI) in mid-pregnancy. This is in line with Obel et al. (2005) who found a trend for low morning cortisol around 14 weeks of gestation in women reporting 1 or more SLEs (Obel et al., 2005). Since only one morning sample was collected (at about +30min post-waking) and awakening cortisol levels were not measured, it is unclear to what extent this reflects morning cortisol activity. Alternatively, Pluess et al. (2010) found no association between SLEs and morning cortisol levels or the CAR area under the curve (AUCg) in early pregnancy, but in late pregnancy there was a negative correlation between SLEs and cortisol levels at 30 minutes post-waking. One other study combined CRISYS-R-neg scores with other stress measures to create a cumulative stress index and found that at 28 weeks gestation, Black and Hispanic women with high levels of cumulative stress had lower cortisol at awakening and at 1.5 hours post-waking compared
to those with low or medium levels of cumulative stress, although no difference in the CAR-MI was seen (Suglia et al., 2010). Thus, overall, the blunted CAR-MI in association with SLEs observed here is in line with the limited body of literature describing low morning cortisol during pregnancy in women experiencing life stressors. Given that an association between a flatter CAR at 23 ± 9 weeks gestation and a shorter length of gestation has been described (Entringer et al., 2011), further studies are needed to corroborate this finding.

Alternatively, perceived stress in mid-pregnancy was not associated with either measure of the CAR or with either measure of the cortisol diurnal profile. Previous studies have also failed to find a relationship in early- and/or mid-pregnancy between PSS scores and the CAR AUCg (Bolten et al., 2011; Pluess et al., 2010) or salivary cortisol levels throughout the day (Spicer et al., 2013). Other studies that utilized single samples of saliva or blood to characterize cortisol levels and also found no association with PSS scores in early- or mid-pregnancy (Baibazarova et al., 2013; Davis & Sandman, 2010; Harville et al., 2009; Ruiz et al., 2001). Even in women experiencing significant psychological distress in mid-pregnancy following the diagnosis of fetal anomaly, no correlations were seen between psychological measures and cortisol levels (measured with a single sample) (Kaasen et al., 2012). It may be that questionnaires assessing anxiety are more appropriate measures to detect alterations in 1st and 2nd trimester cortisol since both maternal state (Sarkar et al., 2006) and trait (Pluess et al., 2010) anxiety have demonstrated associations with prenatal cortisol.

Likewise, late pregnancy cortisol measures were unrelated to stress variables (perceived stress and SLEs), reflecting what others have found (Spicer et al., 2013; Pluess et al., 2012; Bolten et al., 2011; Kivlghan et al., 2008). Alternatively, some previous studies have found associations between cortisol measures in the 3rd trimester and perceived stress (Davis & Sandman, 2010), psychological distress (Voegtline et al., 2013), stressful life events (Obel et al., 2005), and clinical depression (O'Keane et al., 2011). Unfortunately, the present result does not add to the literature because analyses were severely underpowered due to the
limited sample size. As Miller et al. (2007) have pointed out, a sample size of 160 is needed to have enough power to detect an effect of moderate size (which is what previous studies of the association between stress and cortisol have found). More large-scale studies of maternal cortisol in late gestation in relation to psychosocial variables are needed, particularly since it has been suggested that during the latter half of gestation vulnerability of the fetus to the effects of maternal cortisol could be increased (Zijlmans et al., 2015a).

Overall, most studies have found no association between various measures of prenatal stress, SLEs, depression, general anxiety, and pregnancy-specific anxiety and maternal cortisol at multiple time points during pregnancy (Hompes et al., 2013; Spicer et al., 2013; Davis & Sandman, 2010; Goedhart et al., 2010; D’Anna et al., 2009; Harville et al., 2009; Shea et al., 2007) as described recently by Zijlmans et al. (2015a) in their systematic review of the association between prenatal cortisol and child outcomes. However, the heterogeneity in measures of maternal distress and inconsistencies in the characterization of HPA axis activity (blood versus saliva, single versus multiple daily samples) challenges comparisons across studies and limits drawing any major conclusions.

**Prenatal Maternal HPA Axis Activity and Infant Allergic Outcomes**

Comparing prenatal maternal cortisol measures between infants with and without a positive SPT response, the CAR AUCg in mid-pregnancy was significantly lower in infants with a positive SPT. However, this association only approached significance when controlling for covariates and the regression model contained interaction effects. There was also a trend for infants with a diagnosed allergic disease to be born to mothers with a lower CAR mean increase in mid-pregnancy. But controlling for covariates nullified this result and again the final model contained interaction effects. Thus, lower morning cortisol levels and activity in mid-pregnancy were modestly associated with infant atopy and allergic disease although these results are highly preliminary and should be regarded as tentative.
Few studies have examined prenatal cortisol in relation to allergic outcomes in early life and of those conducted to date, all measured cortisol in late pregnancy. These studies found blunted diurnal cortisol in late pregnancy was associated with asthma-related outcomes across the first two years of life (Wright et al., 2013; Beijers et al., 2010) and high levels of mid-day cortisol in late pregnancy were associated with more allergic reactions in the first 4 months of life (although this association was not significant possibly due to a small sample size) (Zijlmans et al., 2015b). These results are difficult to compare to the present results due to substantially different methodologies (e.g., differing cortisol measures, type of allergic outcome, etc.) but it is worth noting that blunted prenatal cortisol activity is common to three out of the four studies. The present study is the first to demonstrate that this association may be seen in CAR measures in addition to diurnal measures, although the small sample size limited the statistical power and the ability to control for multiple confounders.

**Prenatal Maternal HPA Axis Activity and Mediating Variables**

Controlling for covariates, the diurnal slope in mid-pregnancy was lower in women reporting financial hardship (the diurnal AUCg was also lower; however, the regression model showed interaction effects suggesting this result is not reliable). This is consistent with Corwin et al. (2013) who found a significant difference in the temporal pattern of cortisol across the day in 3rd trimester pregnant women who did and did not receive government financial assistance. Although the diurnal profile was not calculated, the raw data showed that the low income group had lower awakening levels and higher evening levels (which would result in a flatter diurnal slope). Alternatively, family income was not associated with the diurnal cortisol slope in early-, mid-, or late-pregnancy in a group of low-income women of Mexican descent (D'Anna et al., 2012). This finding is less relatable to the present study; however, given that the range of family income in this sample was narrow (i.e., all were low-income) and the slope here was calculated as change from morning to afternoon only but excluding an evening value from diurnal slope calculations.
substantially alters the diurnal slope value. The significant association found in the present study between a blunted prenatal diurnal slope and financial hardship warrants further study.

Studies in non-pregnant populations have been inconsistent regarding the association between income/SES and the diurnal cortisol slope. Two large-scale prospective studies found low income was associated with a lower diurnal slope (Hajat et al., 2010; Cohen et al., 2006a) and one study in low-income women found that high levels of material hardship were associated with a blunted morning cortisol response and a trend for higher evening cortisol levels (Ranjit et al., 2005). Despite the latter result being only marginally significant, it is noteworthy in that material hardship was defined in a similar way to the definition of financial hardship used in the present study; namely, by assessing the experience of certain relatively extreme forms of financial stressors in the previous 12 months (e.g., gas or electricity shut-offs, affordability of basic resources such as clothing). However, other studies have failed to find a difference in the diurnal cortisol profile by income/SES (Clearfield et al., 2014; Karlamangla et al., 2013; Cohen et al., 2006b), suggesting more research is needed to better understand the context in which income may affect HPA axis activity.

This result, and the previous finding of a blunted CAR in pregnant women experiencing stressful life events, is also significant in light of previous reports of low cortisol levels and/or hyporeactivity of the HPA axis in patients with post-traumatic stress disorder (PTSD) (Yehuda et al., 2005), depressed women on job-stress-related long-term sick leave (Wahlberg et al., 2009), depressed pregnant women with a history of childhood maltreatment (Shea et al., 2007), and other stress-related disorders (e.g., fibromyalgia, chronic fatigue syndrome) (Heim et al., 2000; Fries et al., 2005). Based on animal data, it has been suggested that chronic stress may induce an extended period of HPA axis hyperactivity that eventually results in hypocortisolism (Fries et al., 2005). This underactivity of the HPA axis is thought to represent an adaptive mechanism whose purpose is to
protect the individual from potentially deleterious effects of glucocorticoid over-exposure (Fries et al., 2005). However, the ramifications of this hypocortisolism are not completely understood, especially within the context of maternal-fetal bidirectional influence.

There was a trend for a lower diurnal slope in participants originating from Africa compared to those originating from West Asia; however, controlling for covariates decreased the association to non-significance and resulted in a model with interaction effects (suggesting this result must be taken tentatively). Racial differences in prenatal cortisol levels have been reported previously with higher levels in Hispanic (Ruiz et al., 2001) and minority (Corwin et al., 2013) women compared to Caucasian women, although one study found lower cortisol in African American women compared to non-Hispanic white women (Glynn et al., 2007). The only study to date to compare the pattern of prenatal cortisol levels throughout the day between racial/ethnic groups is that of Corwin et al. (2013), who found no difference between minority women compared to Caucasian women. Unfortunately, however, the grouping together of women into a single ‘minority’ group may have masked differences between ethnicities or between cultural practices within ethnic groups.

Preliminary evidence shows that psychosocial factors unique to these populations may affect cortisol activity and/or may operate differently in various racial/ethnic groups. Suglia et al. (2010) found a high level of cumulative stress was associated with a flatter diurnal profile in Black women but not in Hispanic women. In women of Mexican descent, more acculturation was associated with a flatter diurnal slope in late pregnancy, but not in early- or mid-pregnancy (D’Anna et al., 2012). Overall, research on prenatal salivary cortisol activity in persons of diverse racial/ethnic groups is extremely scarce and very few studies have examined differences between or within racial/ethnic groups. In non-pregnant populations, Black and Hispanic individuals show a flatter diurnal slope compared to White individuals (Karlamangla et al., 2013; Fuller-Rowell et al., 2012; Skinner et al., 2011; Hajat et al., 2010; Cohen et al., 2006a); however, little is known about
diurnal salivary cortisol in individuals from other ethnic backgrounds. Therefore, the finding here of a smaller cortisol decline across the day in mid-pregnancy in participants from Africa compared to those from West Asia, though preliminary, makes an important contribution to the existing body of research on salivary cortisol.

4.3.2 Infant HPA Axis Activity

The relationship between maternal and infant HPA axis activity observed here was complex and difficult to interpret. In the postpartum, synchrony was observed between maternal and infant diurnal cortisol activity only (i.e., no synchrony was observed between maternal and infant CAR measures). The opposite pattern emerged when examining the association between prenatal maternal HPA axis activity and infant HPA axis activity: there was a highly negative correlation for the CAR-MI. Considering the extremely small sample size and some irregularities in the data (outlined below) the former, but not the latter, result appears to be robust and is discussed in the context of previous research. This result is presented as a trend rather than a finding due to the limited sample size.

Examination of the raw data showed inconsistencies in some measures of infant cortisol between the two sampling days that must be addressed: specifically, the infant CAR-MI was not correlated between days. This suggests that this measure, calculated as the mean between the two days, is not reliable in the present study. This could explain the lack of synchrony between maternal and infant CAR measures in the postpartum and suggests the finding of a negative correlation between prenatal maternal CAR-MI and infant CAR-MI may therefore also not be a reliable result. It seems likely that the sampling protocol may not have been strictly followed and this affected postpartum CAR measures, given the complexity of concurrently sampling one's own and an infant/toddler's saliva at the same time first thing upon waking. Others have noted that the CAR is particularly susceptible to non-compliance (Saxbe et al., 2008) and small fluctuations in sample collection times can alter CAR values (Smyth et al., 2013). For example, a study of preschool children, approximately 3 years old, found latencies in collecting saliva samples were negatively
correlated with morning cortisol levels (Zalewski et al., 2012). Additionally, others have pointed out that CAR-related saliva sampling in young children by parents may be delayed if the child wakes earlier than the parent (Gribbin et al., 2012).

Cortisol circadian rhythms are evident in infant saliva as early as 2 months of age and despite wide variation in the age of appearance and stability of this rhythm (De Weerth et al., 2003), by 6 months of age diurnal variation in cortisol levels is relatively stable (Lewis & Ramsay, 1995). The infant diurnal slopes were somewhat correlated between days; however, caution is warranted in interpreting results since the slope value takes into account waking levels which, as stated, are affected by non-compliance. The AUCg measures reflect total hormonal output, as opposed to responsivity of the HPA axis reflected in the CAR-MI, and these appeared to be more robust to daily fluctuations within individual infants. Together this suggests that the results in the present study worth noting are the synchrony in the postpartum maternal-infant diurnal AUCg (and, to a lesser extent, the diurnal slope).

Only a few studies have examined synchrony in diurnal maternal-infant salivary cortisol levels and these have differed from the present study in the ages of children sampled, timing and frequency of salivary sampling and cortisol measures. Significant correlations between maternal and infant salivary cortisol levels at multiple times across the day have been reported in infants aged 7-17 months (Bright et al., 2012) and in younger infants as well (aged 6 months) (Stenius et al., 2008). One other study with infants aged 4-11 months found correlations with maternal cortisol levels only at bedtime (Benjamin Neelon et al., 2015). Present results are also in line with previous studies that found synchrony in maternal and infant diurnal AUCg, although only in infants under 12 months of age (Bright et al., 2012), and in synchrony in maternal and infant diurnal slope (Stenius et al., 2008).
These analyses controlled for a number of confounders (wake time, infant age etc.); however, previous studies have noted an impact of other variables on maternal-infant synchrony which should be explored in future studies. For example, Clearfield et al. (2014) found synchrony in maternal and infant (6-12 months of age) salivary cortisol at morning and evening only in high SES dyads while low SES dyads were not correlated at any time of day. Breastfeeding was shown to affect maternal-infant salivary cortisol synchrony at nighttime in one study (Benjamin Neelon et al., 2015) but no effect was seen on cortisol levels or diurnal slope in an earlier study (Stenius et al., 2008). It is noteworthy that a significant correlation was seen here between maternal-infant measures of diurnal cortisol despite the lack of controlling for these factors.

It is unfortunate that so few infant salivary samples were collected and this study cannot address the extent to which prenatal maternal cortisol and/or psychosocial factors contribute to infant cortisol activity. However, previous reviews have noted the many difficulties inherent in both the collection and analysis of infant/toddler salivary cortisol including the influence of sleeping and feeding (Tryphonopoulos et al., 2014; Egliston et al., 2007). One study found that eating within a half hour of sample collection occurred in 10-20% of cases and was associated with lower cortisol levels (Zalewski et al., 2012). A recent study of toddlers between the ages of 30-36 months found a significant influence of the presence and timing of napping on the pattern of diurnal cortisol (Tribble et al., 2015). Studies with larger sample sizes and monitoring of protocol adherence may help clarify the predictors of infant HPA axis activity and their role in childhood health.

4.3.3 Prenatal Maternal Immune Factors

High correlations between cytokines were seen here, as others have found. Furthermore, the magnitude of the correlations closely resembled those reported by Curry et al. (2008) from a large Danish cohort study of cytokines levels (by multiplex flow cytometric assay) in early and mid-gestation and those reported by Cassidy-Bushrow et al. (2012) in a study of 187 African American women during the 2nd trimester. Some caution is warranted in
comparing results across studies that measured cytokine levels at different stages of pregnancy, however, because some cytokine levels vary across gestation while others do not (Holtan et al., 2015; Ferguson et al., 2014; Azar & Mercer, 2013; Denney et al., 2011; Kraus et al., 2010; Curry et al., 2008; Vassiliadis et al., 1998). Nevertheless, the consistency of cytokine correlations between the present study and those previously reported strengthen confidence in cytokine-related results of the present study.

A trend was found in the present study for lower serum IFNγ:IL-4 in late pregnancy in mothers of infants with allergic sensitization, similar to the finding reported by Kim et al. (2008) who found low mid-pregnancy IFNγ:IL-4 was associated with atopy in children at the age of 3. This is the only other study to date to examine prenatal cytokine ratios in relation to atopy in the offspring. An important consideration in interpreting these findings is that peripheral maternal cytokine patterns do not necessarily reflect the environment at the fetomaternal interface, nor within the placenta or fetus (Pfefferle et al., 2011). Nevertheless, maternal prenatal immune factors including cytokines and co-stimulatory molecules affect the development of neonatal immunity, including establishment of Th cell subset profiles (Basha et al., 2014).

There is growing interest in the role of prenatal maternal immunity in obstetric outcomes and infant health; however, more research is needed to understand the normal ranges of cytokines and other immune markers, the mechanism by which these factors influence the development of the infant’s immune system, and the contribution made by various sources (maternal serum, placenta, amniotic fluid, breast milk) (Agarwal et al., 2011).

A borderline trend was observed here for a positive correlation between prenatal IL-10 and the experience of financial hardship during pregnancy. This is not consistent with previous studies of IL-10 in relation to prenatal distress, although the number of prior studies is limited. Furthermore, no previous studies measured these variables in late
pregnancy and whether IL-10 levels measured at different stages of pregnancy may be compared between studies is not clear because across gestation no change (Holtan et al., 2015; Ferguson et al., 2014; Denney et al., 2011) and increasing levels (Brogin Moreli et al., 2012) of IL-10 have both been reported. Nevertheless, previous research has found no association between stress and IL-10 in mid-pregnancy (Shelton et al., 2015; Coussons-Read et al., 2007), a negative correlation in early-pregnancy (Coussons-Read et al., 2007), and a negative correlation in early- to mid-pregnancy that was not seen in the 3rd trimester (Latendresse et al., 2013). One other study found that depressive symptoms were associated with higher IL-10 in leaner women at mid-pregnancy while the opposite pattern was seen in heavier women (Cassidy-Bushrow et al., 2012).

IL-10 is often classified as a Th2 (anti-inflammatory) cytokine, but more recently is described as neither a Th1 nor Th2 cytokine because it may act to either stimulate or inhibit immune activity depending on the context (Brogin Moreli et al., 2012). IL-10 is produced by many cells of both the innate and adaptive immune system and is involved in feedback regulation of numerous types of immune responses (Saraiva & O'Garra, 2010). The well-described actions of IL-10 include the inhibition of both Th1 and Th2 responses, and the induction of its own production in a positive feedback loop via increased differentiation of IL-10-secreting Treg cells (Saraiva & O'Garra, 2010). During early pregnancy, IL-10 production at the fetomaternal interface plays an important role in the establishment and maintenance of pregnancy (Sykes et al., 2012). However, elevated IL-10 in maternal circulation later in pregnancy has been associated with PTB (Ferguson et al., 2014; Cassidy-Bushrow et al., 2012; Pearce et al., 2010). Therefore, the finding here of higher IL-10 in late pregnancy in women with financial hardship, while highly preliminary and not statistically significant, is nevertheless worthy of replication.

No associations were seen here between any of the cytokine levels in late pregnancy and perceived stress or SLEs. This is in line with previous research showing no relationship between prenatal maternal stress and serum cytokines in early- to mid-pregnancy.
(Christian et al., 2009), mid-pregnancy (Shelton et al., 2015), and late pregnancy (Cheng & Pickler, 2014). Others observed an association between cytokines and maternal stress in early but not mid-pregnancy (Coussons-Read et al., 2007), at mid- but not in late pregnancy (Latendresse et al., 2013) or in early and late pregnancy (Coussons-Read et al., 2012); however, conflicting associations were described between these studies. Prenatal depressive symptoms have been studied more extensively in relation to prenatal cytokine levels; however, results of these studies have also been mixed. Several studies found depressive symptoms were associated with elevated proinflammatory cytokines (Azar & Mercer, 2013; Latendresse et al., 2013; Cassidy-Bushrow et al., 2012; Christian et al., 2009; Ruiz et al., 2007) while others failed to find an association between depressive symptoms and maternal cytokines during pregnancy (Blackmore et al., 2014; Cheng & Pickler, 2014; Okun et al., 2013; Blackmore et al., 2011). Conflicting results likely stem from differences in sample characteristics, stress measures, and the timing of cytokine measurement. Nevertheless, the relationship between psychological states (stress, depression etc.) and cytokines during pregnancy remains unclear but will likely be better defined in the coming years as this topic continues to garner increased research attention due, in part, to more accessible and less costly cytokine analyses. This research is needed, given preliminary evidence suggesting cytokines may mediate the relationship between PNMS and poor birth outcomes (e.g., GA at birth) (Coussons-Read et al., 2012).
5 Study Strengths, Limitations & Future Directions

The present study has several strengths and weaknesses which will be discussed in turn. Specifically, the inclusion of non-English-speaking women, the use of an objective measure of atopy in both mother and child, and the multiple measures of prenatal stress and potential underlying mechanisms make this study unique and valuable. On the other hand, the small sample size (with a large portion subsequently lost to follow up), and lack of both a control group and of salivary sampling monitoring contribute to the need for cautious interpretation of results. Overall, this pilot study was useful in highlighting key issues surrounding data collection in this kind of population that must be addressed in future studies.

Study Strengths

A major strength of the present study is the inclusion of women with a language barrier. In the largest study to date comparing prenatal depressive symptoms and associated risk factors in Canadian-born and immigrant women, Miszkurka et al. (2012) acknowledged that 33% of eligible participants were excluded due to the presence of a language barrier. This represents a substantial portion of women whose experiences were not captured in their research. A language barrier is known to contribute to stress, social isolation, and depression (Guruge et al., 2009). In female new immigrants to Canada, limited language proficiency that persists over time is also associated with declining self-reported health (Ng et al., 2011). Together, this suggests that excluding female immigrants with a language barrier may lead to underestimation of stress/depression prevalence and an incomplete picture of the resulting consequences in the immigrant population.

Furthermore, as in immigrants to other countries, in Canadian immigrant women, a language barrier negatively affects the experience of health care including access to and receipt of proper care (Guruge et al., 2009; Reitmanova & Gustafson, 2008). Thus, recruiting women from the community (rather than via health care access points) and
including those with a language barrier may result in a more representative sample of the pregnant immigrant population and this may inform better clinical practice and, ultimately, better health care for these women. A language barrier is an obstacle to the recruitment of ethnic minorities into mental health research (Brown et al., 2014); however, Canadian researchers should not shy away from this undertaking despite its many inherent challenges.

Another major strength of the present study was the inclusion of an objective measure of infant atopy (i.e., allergic sensitization via SPT) in addition to maternal reports of physician-diagnosed allergy. Sensitization measured by SPT is associated with an increased risk for asthma and allergic disorders in children (Kim et al., 2013; Peroni et al., 2008; Kurukulaaratchy et al., 2005; El-Sharif et al., 2003; Soto-Quiros et al., 2002) and is superior to sensitization measured by specific IgE in predicting the development of allergic disease in childhood (Ro et al., 2015; Kjaer et al., 2009). There is also evidence that a positive SPT response in the first year of life and other markers of early sensitization predict asthma in adulthood (Arshad et al., 2005; Martinez, 2002; Rhodes et al., 2001). Transient SPT reactivity across the first 18 months of life occurs with some frequency, suggesting some caution is warranted in interpreting SPT results before the age of 2 (Bernstein et al., 2008); however, even transient SPT responses are associated with allergic disease at this age (Johnke et al., 2006). Some stability in sensitization across the early years of life highlights the fact that early identification of this phenotype yields potential for early intervention (Dean et al., 2007).

Here the standardized cut off of ≥3mm wheal diameter (relative to negative control) was used to define atopy (Eigenmann et al., 2013; Bernstein et al., 2008) but 2 infants with a ≥2mm wheal diameter plus symptoms suggestive of allergic disease were also included. Other authors have used this ≥2mm cut-off to investigate the prevalence of allergic sensitization or the association between sensitization and allergic disease in infants/children (Almqvist et al., 2007; Johnke et al., 2006; van Amsterdam et al., 2004;
Arshad et al., 2001; Arshad et al., 1993). In at least one study, this lower wheal cut-off value (≥2mm versus ≥3mm) was better at predicting persistence (i.e., a positive SPT response to an allergen that was never followed by a negative response) which was, in turn, associated with allergic dermatitis (Johnke et al., 2006). Additionally, the expert guidelines on the diagnosis of IgE mediated allergies in children states a combination of clinical history (i.e., symptoms), physical examination, and validated allergy tests should be used (Eigenmann et al., 2013). Thus, while a physical examination of all infants in the present study was not possible, the addition of symptoms to the definition of atopy adds an important clinical element.

An objective measure of atopy (i.e., allergic sensitization measured by SPT) was also conducted in the mothers and included as a covariate in the main analyses. Previous research has shown that a positive SPT in the mother or father is associated with sensitization and atopic dermatitis in infancy (Johnke et al., 2006) and that maternal atopy may moderate the effect of prenatal life stress on cord blood IgE levels (Peters et al., 2012). Considering that there is a large hereditary component to atopy and allergic disease development, with the maternal contribution being particularly strong (Yung et al., 2015; Soto-Quiros et al., 2002), maternal atopy is an important covariate in these types of analyses. SPT responses could be considered the most appropriate measure of maternal allergy since self-reported disease is subject to under-diagnosis (perhaps related to language barriers or lack of access to proper care in immigrants) and total IgE, while related to allergic sensitization, varies widely in the general population (Kerkhof et al., 1996).

The rate of maternal sensitization via SPT found here (35%) closely resembles the rate reported for Swedish adults (33%) (Plaschke et al., 1996) but lower than that of a sample of young adults in Brazil (47.6%) (Ferraz et al., 2011). House dust mite is often the most common allergen to show sensitization in adults (Ferraz et al., 2011; Kerkhof et al., 1996) and children (Kim et al., 2013; Kurukulaaratchy et al., 2005) although a high rate of
sensitization to cat has also been previously reported (Plaschke et al., 1996). Here, cockroach sensitization was as common as sensitization to dust mite (both $n = 11$), followed by dust and cat (both $n = 9$). Skin prick testing is usually performed only in cases of suspected atopy and therefore information on the sensitization rates, in general and in response to specific allergens, in the general population is sparse. Nevertheless, maternal SPT responses in this sample of ethnically-diverse immigrant women are comparable to previously reported rates in adults although further research should explore the relatively high rate of sensitization to cockroach in this group of women.

Another major strength of the present study is the multiple measures of stress utilized. Both stressful life events and perceived stress were included because while they are correlated, they have different risk factors (Kingston et al., 2012) and may have different consequences for maternal and child health. Previous studies of PNMS programming of immunity have traditionally used only one measure of stress (Hartwig et al., 2014; Chiu et al., 2012; deMarco et al., 2012; Khashan et al., 2012; Fang et al., 2011; Sernthial et al., 2011; Kozyrskyj et al., 2010; Cookson et al., 2009; Sausenthaler et al., 2009) or a composite measure (Guxens et al., 2014; Reyes et al., 2011; Wen et al., 2011), the latter of which may mask subtle differences between stress types. One notable exception is the study by Turcotte-Tremblay et al. (2014) who measured both objective and subjective responses to the Quebec Ice Storm of 1998.

Stress in the present study was also measured at multiple time points, both early/mid-pregnancy and late-pregnancy, while most previous studies only looked at one time point (with the exceptions of Hartwig et al., 2014 and Cookson et al., 2009). Levels of prenatal distress have been shown to change over the course of pregnancy (Rallis et al., 2014; Woods et al., 2010) as have maternal responses to stressful events (Glynn et al., 2001). Furthermore, it remains unclear where the ‘window of vulnerability’ for prenatal immune programming occurs. In animal studies the timing, type, and duration of PNMS had differential effects on the programming of offspring immunity (Veru et al., 2014; Merlot et
al., 2008). Thus, human studies exploring this hypothesis should employ a thorough assessment of PNMS (namely, measurement of objective and subjective stress throughout the course of pregnancy) to facilitate a more complete picture of the effects of PNMS on immune development in the offspring.

Finally, another major strength of this study is the exploration of multiple potential mechanisms underlying PNMS programming of infant atopy. Few studies to date have measured maternal and/or infant HPA axis activity or maternal immunity in relation to atopy or allergy in the next generation, and none have concurrently measured all three. While no major conclusions can be drawn as a result of the limited sample size (discussed in the section to follow), the present study illustrates that this approach is feasible. Future studies employing a similar approach may help clarify the specific impact(s) of stress so that targeted interventions and/or prevention strategies may be designed and implemented to reduce any deleterious effects.

**Study Limitations**

The most substantial limitation of this study is the small sample size which was even further decreased at each subsequent visit, resulting in a very small final sample. This significantly reduced the statistical power and limited the chance of detecting an effect in several of the analyses, but especially in those involving visit #3 data. However, given that the overall aim of this study was to assess the feasibility of our protocol, we knew at the outset that the results would be underpowered and would therefore need to be considered exploratory. For example, using the adjusted OR for atopic dermatitis at age 2 in association with prenatal psychological distress described by Wen et al. (2011) (OR = 2.3; 95% CI, 1.1 to 5.3) a sample size of 371 would be needed to detect an association with a 95% confidence level.
At visit #2, 73% of the original sample was retained and by visit #3 while only 44% of the original sample remained, this fraction represented 61% of those who attended visit #2. Thus, most participants who were lost to follow up were lost relatively early in the study. This low retention rate is not unusual in cohort studies. For example, in the Avon Longitudinal Study of Parents and Children (ALSPAC Study), the participation rate was 56% at the 10 year follow-up visit (Howe et al., 2013). Compared to the ALSPAC study, the follow-up period here was considerably shorter (2 years on average, but up to 3 years for some participants); however, the population under investigation was also quite different. Psychosocial distress has been suggested as a barrier to retention of minority persons in research studies (Yancey et al., 2006). In line with this, a trend was seen here indicating women who did not attend the final study visit had more depressive symptoms in late pregnancy (see Appendix H, Tables H1). Furthermore, these women were significantly less likely to be working or attending school during early-/mid-pregnancy compared to those who persisted for the entire study (Appendix H, Table H2). This may indicate that women who were not socially engaged, or perhaps did not have access to transportation and/or child care, and had more psychological distress during pregnancy were less likely to engage in the complete study protocol. Considering immigrant women tend to face these issues more often than non-immigrant women, further research should explore strategies to retain immigrant women in longitudinal research studies.

There remains a clear lack of information on successful techniques to recruit and retain participants from diverse racial/ethnic backgrounds (Napoles & Chadiha, 2011). In general, studies have focused mostly on recruitment rather than retention strategies for minority persons (Yancey et al., 2006). This issue deserves more study, particularly in immigrant populations, since lower SES (Odierna & Bero, 2014; Yancey et al., 2006) and parity (Abdou et al., 2010) have been cited as barriers to study retention and these factors may be more common in immigrant women. Many longitudinal and birth cohort studies cite lower SES in participants lost to follow up compared to those persisting (e.g., Howe et al., 2013; Abdou et al., 2010; Morgan et al., 2005; Koopman et al., 2002; Arshad et al., 2001). Of particular interest, this may lead to underestimation of socioeconomic inequalities in
birth outcomes (e.g., PTB, birth weight) (Howe et al., 2013). Recruiting and retaining minority and/or low SES participants in research studies has been a challenge that needs to be addressed in future studies (Wegienka et al., 2013).

The small sample size also had a negative impact on the salivary cortisol analyses, especially those involving infants (which were part of the study’s secondary aims), because fewer participants completed the saliva sampling protocol than completed study visits. Some insight into the reason for the loss of some saliva samples comes from one qualitative study about participants’ comfort level and attitudes toward their participation in a prenatal cohort research study. Daniels et al. (2006) found that providing saliva samples was the most troublesome feature of their research protocol, which additionally involved questionnaires, ultrasounds, and blood sampling (the latter of which is considered more invasive). In the present study, several participants had medical issues preventing them from participating in the saliva sampling and some simply stated they were ‘too busy’ to complete the sampling. It is possible that the collection of six samples per day across two consecutive days was too burdensome and future studies should consider minimizing the number of samples collected (discussed further in section 4.3).

Additional salivary samples in infants, toddlers and children are often lost due to insufficient quantity of saliva collected (Ivars et al., 2015; Baumler et al., 2013) as was the case in the present study as well. Thus, most cortisol analyses were underpowered here and visit #3 cortisol analyses were severely underpowered, meaning ultimately these cannot add to the literature of whether infant salivary cortisol acts as an underlying mechanism in the PNMS programming of immunity.

Another potential issue with the analyses relating perceived stress to cortisol measures is that the stress measure reflected participants’ recall of their experience over the previous two weeks. Some research has found significant links between mood and cortisol
measures using ecological momentary assessment and cortisol sampling, including during pregnancy (Giesbrecht et al., 2012). At least one prior study found that, during pregnancy, a substantial portion of the variance in diurnal cortisol was accounted for by within-person changes in negative mood (Giesbrecht et al., 2012). This suggests that concurrent measurement of negative mood and cortisol during pregnancy is needed to better understand associations between emotional/behavioural processes and biological systems.

One final limitation regarding cortisol analyses is that compliance with the timing of salivary sampling was not monitored. The exact timing of saliva collection is known to greatly influence cortisol measures, especially the CAR (Saxbe et al., 2008) but also the diurnal cortisol profile (Moeller et al., 2014) and this is an issue for infant samples as well (Tryphonopoulos et al., 2014). Monitoring of sampling times would also eliminate the problem encountered in the present study of missing diaries. Here, mean values of the whole group were used when saliva samples were provided without diaries, an event that occurred at a relatively low rate (5/57 or 8.8%). Nevertheless, the extent to which this may have influenced the results is not known and must be considered a limitation. Moving forward, objective information on the timing of saliva sampling will be fundamental in distinguishing whether blunted salivary cortisol levels and/or responses are the result of psychosocial factors (e.g., low income, non-Caucasian ethnicity) or noncompliance with ambulatory salivary sampling protocols.

And, finally, the design of the present study could be considered a limitation inasmuch as it was supposed that Canadian immigrant women represent a group with higher rates of prenatal stress and/or greater exposure to chronic stress, but this wasn’t formally tested. Including a sample of pregnant Canadian-born women from diverse ethnic and cultural backgrounds may have clarified whether immigrant women experienced greater prenatal stress, but this was not the study focus. Alternatively, a larger sample size would have allowed the division of participants into highly stressed and non-stressed groups so that
the role of prenatal stress on infant atopy could be examined in a group of immigrants without the assumption that immigration is equated with stress.

Without the inclusion of a non-immigrant comparison group, it is also difficult to account for differences in the rates of allergic sensitization and diagnosed allergies relative to other studies. Here the rate of a positive SPT response using a 3mm cut-off was 16%, but a rate of 6-11% at 18-24 months of age has been reported previously (Ro et al., 2015; Johnke et al., 2006). The presence of a physician-diagnosed allergy found here (13.8%) is significantly lower than a recent population-based study where the rate of allergic disease at age 2 was 25.6% (Ro et al., 2015). It is possible that the small sample size accounts for these discrepancies; however, psychosocial, lifestyle and/or environmental factors cannot be discounted as potential contributors.

Lessons Learned & Future Directions

A major focus of the present study was to examine the feasibility of testing this hypothesis in this type of population. In doing so, we hoped to describe the barriers and facilitators encountered along the way to inform future studies. First, the success of the recruitment and retention strategies utilized here (i.e., lessons learned) are discussed in relation to those used in similar populations. This forms the basis for recommendations on future studies of ethnically-heterogeneous samples of women, particularly immigrant women. Next, suggestions are provided for future studies of PNMS programming of immunity in light of results found here and results of other research in this field.

Several recent reviews have summarized the barriers in recruiting persons from diverse racial/ethnic backgrounds (for example, see Brown et al., 2014; Ibrahim & Sidani, 2014); however, consensus on the most effective solutions to these obstacles remains an area of
active study. Some have argued that funding initiatives that focus specifically on factors that contribute to successful recruitment of ethnic minority groups should be created; ...These initiatives are especially indicated in the case of large population-based cohort studies involving underrepresented groups, where biological specimens or burdensome procedures are required that can impose particular hardships on participants. (Napoles & Chadiha, 2001; page S145).

Nevertheless, previous studies provide some useful information that may be applied to the recruitment of immigrant women living in Canada, a group spanning many different source countries and ethnicities.

According to a recent systematic review, both proactive and reactive approaches to recruitment are effective in recruitment of minority individuals into research studies (Ibrahim & Sidani, 2014); however, the costs and yield may vary considerably (Yancey et al., 2006). Here, a convenience sample was recruited via (in order of effectiveness) in-person recruitment efforts by community liaisons and the student, snowballing (i.e., word of mouth) and advertisements. These techniques are commonly used in studies of immigrants (Lopez-Class et al., 2015) and minority persons (Ibrahim & Sidani, 2014). Other community-based longitudinal studies have also found that face-to-face is the most effective means of recruitment, especially for recruitment of participants who remain in the study over time. Advertisements and printed materials (i.e., fliers) provide a higher yield of respondents but also a higher rate of non-eligibility and attrition (Gillis et al., 2001). The main drawback to this approach is that a self-selection bias may make results less generalizable to all immigrants (Lopez-Class et al., 2015) but a self-selection bias may also occur following other types of recruitment. Studies of mental health issues often recruit participants from clinical sites; however, this may be problematic in this population considering that ethnic minorities are known to underutilize mental health services. Thus, recruiting from clinical sites may also provide a biased sample by recruiting only the fraction with mental health issues who also seek professional help (Brown et al., 2014). Overall, recruitment through the community is likely the best way to acquire a
representative sample, provided care is taken in accommodating individuals who are typically less likely to participate in research (e.g., those with language/cultural/religious barriers, mobility issues etc.) as described below. Thus, the sample studied here is likely representative of the local immigrant community, but not necessarily a 'highly stressed' group.

The importance of employing a community-based approach has been acknowledged by several authors (Lopez-Class et al., 2015; Wegienka et al., 2013; Yancey et al., 2006). Here, working with community centres that offered free prenatal health classes was the most fruitful avenue for finding and recruiting participants, although community midwife centres were also helpful. In particular, the formation of an advisory group made up of lay persons from within the community is thought to be central to gaining key information about, and access to, a specific population (e.g., specific cultural values or practices) (Lopez-Class et al., 2015; Yancey et al., 2006). This technique worked well in the present study highlighting certain exposures in the home that may be relevant to the development of allergies which were then included in participant questionnaires. For example, in the Muslim religion, dogs are considered unclean and are therefore not commonly kept as pets; however, the burning of incense is a common (sometimes daily) practice in many Islamic homes.

Alternatively, researchers must be aware that other issues may not be identified prior to the start of the study despite the employment of a community-based advisory group, but rather emerge organically during study visits. For example, while it is generally accepted that pregnant or breastfeeding women are exempt from fasting during the daylight hours of the month of Ramadan, some of these women nevertheless choose to partake in fasting. These kinds of issues may have consequences for studies collecting biological measures (e.g., salivary cortisol) and may therefore require protocol flexibility and/or creative problem-solving as they emerge.
Another important strategy utilized here, as well as in other longitudinal community-based studies of minority women, was gender- and ethnicity-/culture-matching staff to participants for recruitment and maintaining participant contact over time (Ibrahim & Sidani, 2014; Gillis et al., 2001). Culture-matching helps establish trust and comfort which, in turn, facilitates open communication from participants (Ibrahim & Sidani, 2014). Religion, mistrust, and stigma are common barriers that may negatively affect both recruitment and disclosure of information (Brown et al., 2014; Gardner et al., 2014) but these are more easily overcome through discussions with other members of their ethnic community who may be viewed as more sympathetic to these issues. This highlights another important element to success, namely employing flexible and personable staff members who establish a rapport with participants (Brown et al., 2014; Ibrahim & Sidani, 2014). Some additional obstacles to recruitment encountered in the present study as well as in previous studies include the fear of being reported to immigration or other government agencies, and incongruence between the wishes of participants and their husbands (Brown et al., 2014; van Delft et al., 2013). Gender- and culture-matched, sensitive staff that is simultaneously invested in both the research and the community may be indispensable in addressing these issues.

In the present study, monetary incentives, childcare and reimbursement for transportation costs were provided as these have been shown to encourage research participation in immigrants and other ethnic minority groups (Lopez-Class et al., 2015; Brown et al., 2014; Odierna & Bero, 2014; Yancey et al., 2006). Motivation to participate in research for some participants is related to personal gains (e.g., informational support, money) while others emphasize a desire to help advance science and/or to help others in their community (Lopez-Class et al., 2015; Odierna & Bero, 2014; Daniels et al., 2006) and this was reflected here as well. Additionally, many women seemed to be thankful that they had someone to talk to about their stresses and grateful that their stories/difficulties were being heard.
Despite all these strategies, recruitment of participants into this study was difficult and time consuming but this is not unique to this study. As Lopez-Class et al. (2015) stated,

...Finally, many studies on immigrants find recruiting their target sample size often leads to extending their project timeline (p. 9).

Recruitment challenges also added to the overall study budget as extra expenses were accrued for reimbursement of recruiters' time and transportation costs (gas, parking, etc.) and for the translation of study fliers and questionnaires into other languages. Future research studies need to be aware of these issues and plan timelines and budgets accordingly, perhaps even setting aside a fraction of the budget for additional (unforeseen) costs that are likely to emerge once the study is under way.

Two other lessons learned are worth mentioning, the first regarding interviewing immigrant women from diverse ethnic backgrounds and the second on retaining these women in a research study over the long term. First, a private location for study interviews is very important in this type of research study. Immigrant women interviewed in their own homes may feel anxious and/or provide less information if their husbands are nearby (Lopez-Class et al., 2015). This also speaks to the need to use study translators, not participant’s family members or friends, in conducting research-related interviews. It is essential that participants feel safe and free to discuss any difficulties in their life and, since stresses are often psychosocial and personal, information must be gathered in a respectful and private manner.

Second, to ensure proper follow up of participants, information on multiple points of contact (e.g., friends, relatives) should be obtained (Lopez-Class et al., 2015; Yancey et al., 2006). Loss to follow up occurs for many reasons including the provision of incorrect contact details (which occurs at a fairly high rate in some women; see van Delft et al., 2013) and relocation. Maintaining frequent contact with participants (e.g., about every 3 months) (Lopez-Class et al., 2015) may minimize loss to follow up due to relocation as well as aiding
in study retention in general (Yancey et al., 2006). Again these strategies increase study costs; for example, multilingual staff who maintain contact with participants with a language barrier are employed for more frequent follow up calls or visits. Prior studies have been hampered by insufficient funds necessary to employ adequate levels of staff in order to schedule visits and sustain contact with participants (Odierna & Bero, 2014).

Some final recommendations that may facilitate future research on immigrant women’s mental health during pregnancy include a) validation of more assessment/diagnostic tools, and b) standardization of the categorization of ethnicities and/or world regions. In the present study, some study materials were translated but not validated, or they were translated by CBRs during interviews as needed. This was largely due to the absence of translated, validated materials. For example, at study commencement the PSS was available in translated versions of several languages (including Arabic); however, a validated version of the Arabic PSS was published only in 2010 (Chaaya et al., 2010) at which time the study had long been underway. Other authors have likewise acknowledged that a lack of translated materials can negatively affect studies of mental health in ethnic minorities, especially culturally-appropriate rather than simple language translations (Brown et al., 2014).

The division of world regions used here differs from other classifications (e.g., Jessri et al., 2013) and this may have obscured some cultural/religious differences in predictors of stress, depression and social support. For example, in the present study Iran was classified under the region of South Central Asia (which included Pakistan, India, and Bangladesh) and Egypt was classified as belonging to the region of Africa; however, the immigrant women in the present study who originated from Iran and Egypt more closely resembled other Muslim women (classified as West Asian) in terms of their lifestyle and traditions. Future studies must be aware of different categorization of world regions as well as different religions and cultures within the same region/country and examine how this may affect the perception and/or reporting of stress.
Suggestions for future studies of PNMS programming of atopy/allergy development in offspring include measuring other types of stress, gathering information on multiple aspects of participants’ behaviours and environmental exposures, and the use of large sample sizes followed over longer periods of time. Furthermore, for studies investigating this hypothesis in immigrants, consideration should be given to disparate study designs. Additionally, recommendations are presented with respect to studies that plan to utilize salivary sampling to examine whether cortisol activity underlies PNMS programming of immune outcomes.

Prenatal distress has been conceptualized in many different ways across programming studies; however, accumulating evidence suggests two kinds of stress, not measured here, may be particularly salient to maternal and/or fetal health: pregnancy-specific stress and perceived discrimination. Pregnancy-specific stress/anxiety has been associated with poor birth outcomes (Coussons-Read et al., 2012; Lobel et al., 2008), elevated levels of maternal cortisol (Kane et al., 2014) and increased levels of maternal inflammatory cytokines (Coussons-Read et al., 2012). Furthermore, pregnancy-specific anxiety has also been associated with infant cortisol reactivity (Tollenaar et al., 2011; Gutteling et al., 2005), possibly via altered methylation of the GC receptor as seen in one study of cord blood (Hompes et al. 2013). Likewise, discrimination/racism is emerging as a potent determinant of mental and physical health (Paradies et al., 2015). Discrimination has been associated with an altered diurnal cortisol rhythm regardless of race (Skinner et al., 2011), while in other studies, specific patterns differed depending on race and SES (Fuller-Rowell et al., 2012). Within the context of programming, discrimination has been associated with both altered prenatal maternal cortisol levels and with infant stress reactivity, independent of ethnicity (Thayer & Kuzawa, 2015). Thus, future research of PNMS programming should further explore pregnancy-specific stress/anxiety and perceived discrimination in addition to other forms of prenatal distress.
Attention must also be paid to better characterization of stress and/or depression in the postpartum period as these are related to subsequent HPA axis activity and mental health (Essex et al., 2011). For example, maternal depressive symptoms are associated with asthma risk during childhood (Kozyrskyj et al., 2008) and, in low income families, are associated with low salivary cortisol and blunted salivary cortisol responses to stress in children age 2-6 years (Fernald et al., 2008). Some, but not all, previous studies of the link between PNMS and childhood allergy have measured and controlled for postnatal stress/depression.

Future studies must carefully consider what aspects of participants’ lifestyle should be monitored and included in statistical analyses as more of these factors emerge as potential confounders. For example, patterns of eating, sleeping, and physical activity have been shown to change for some women under PNMS (Beijers et al., 2014) and this could play a role in the association between PNMS and infant immune outcomes. Children born to mothers with the most weight gain across pregnancy were shown to have altered immune responses early in life (at birth and 3 months of age) and an increased risk of asthma at age 2-9 (Halonen et al., 2013). Furthermore, BMI is associated with SPT positivity in adult women (Skaaby et al., 2015) and BMI/weight during pregnancy has been associated with prenatal cortisol levels in several (Goedhart et al., 2010; Harville et al., 2009; Obel et al., 2005) but not all (King et al., 2010) studies. Measuring environmental exposures such as dust samples and mold in the home is likely another important feature to include in studies of this kind as allergic susceptibility is associated with differing levels and types of allergens. In order to control for such a large number of confounders while retaining adequate power; however, future studies will need very larger sample sizes. Furthermore, follow-up for a longer period of time will aid in discriminating transient allergic features from clinically significant allergic disease.

In order to better understand the role of foreign birthplace, ethnicity and acculturation on PNMS, allergy susceptibility, and/or PNMS programming of atopy in offspring, more
studies are needed with persons from diverse cultural/ethnic backgrounds. Furthermore, future studies should consider disparate, underutilized study designs such as examining rates of allergic disease and changes over time a) within a group of immigrants all originating from the same country or region or b) within one ethnic group, comparing foreign-born versus Canadian-born individuals. Another potentially fruitful study design would be to study multiple children within the same immigrant family where some children were born in Canada while others were born in the family's country of origin. This could help disentangle the effects of certain environmental exposures from the heritable component of allergic disease development while maintaining consistency in other lifestyle/behavioural factors (e.g., diet).

Studies utilizing a salivary sampling protocol should consider measuring and controlling for whether samples were collected on a week day or weekend, the season, participants' level of physical activity, and parity (Karlamangla et al., 2013; Goedhart et al., 2010; Harville et al., 2009; Vreeburg et al., 2009; Kivlighan et al., 2008; Saxbe, 2008) – all previously shown to affect cortisol levels during pregnancy. Consideration should be given to monitoring salivary sampling compliance with MEMScaps, as recommended by Saxbe et al., 2008, especially for infant samples (Tryphonopoulos et al., 2014). While several different measures of HPA axis activity were used, HPA reactivity to a stressor was not assessed – and previous studies have found different effects of PNMS on infant cortisol reactivity depending on the stressor type (vaccination, maternal separation) (Tollenaar et al., 2011).
6 Conclusions

The clustering of risk factors in Canadian immigrant women is both troubling and significant from a public health standpoint, especially in the context of pregnancy. Recruiting and retaining a representative sample of pregnant Canadian immigrants is extremely challenging, expensive, and time-consuming. However, the difficulties inherent in research with this population have created a dearth of information on the causes and consequences of poor mental health in this group, with implications for clinical practice and public policy. This is particularly true of immigrants to Canada, a group that includes one of the most racially and ethnically diverse individuals in the world. Here we show that a biopsychosocial research methodology can be carried out in this group; however, only with the help of community-based researchers who are themselves a group of ethnically-diverse immigrants to Canada. Although limited by a small sample size, lower levels of prenatal perceived stress were associated with a greater likelihood of allergic sensitization in infants and this association was driven by higher SES (i.e., a lack of financial difficulties). A blunted pattern of prenatal HPA axis activity was seen in mid-pregnancy in association with financial difficulties, but to what extent and how this may influence infant atopy remains unclear. Studies examining these questions in a larger sample size may shed
further light on the complex relationship between maternal psychosocial stress and allergy development in children and whether immigration and immigration-related factors uniquely contribute to this relationship. Given the rising rates of allergic disease and the fact that Canada is largely a country of immigrants, such research is urgently needed.
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## Appendix A: Human Studies of Prenatal Stress (PNMS) and Atopic Disease Outcomes in Offspring

<table>
<thead>
<tr>
<th>Authors</th>
<th>Study Design</th>
<th>Sample</th>
<th>Timing of PNMS</th>
<th>Type of PNMS</th>
<th>Outcome</th>
</tr>
</thead>
</table>
| Guxens et al., 2014   | Population cohort (Generation R Study*) | N = 4,848 | 2nd trimester (assessed at 20 weeks gestation) | Psychological distress via the Brief Symptom Inventory (including global index of psychological symptoms, depression scale, and anxiety scale) | PNMS associated with increased odds of wheeze at age 1-4  
|                       |                         |        |                | PNMS associated with increased odds of physician-diagnosed asthma at age 6 (trend)                     |                                                                         |
| Hartwig et al., 2014  | Prospective cohort (Raine study*)     | N = 1,587 | i) 0-18 weeks and ii) 18-34 weeks gestation | 10 common adverse events via the Tennant and Andrews validated life-events instrument                  | PNMS during the 2nd half of pregnancy associated with higher odds of asthma and eczema at age 14, but not at age 6  
|                       |                         |        |                | Trend for PNMS in the 2nd half of pregnancy to be associated with higher odds of rhinitis at age 14 (p = 0.07) |                                                                         |
| Turcotte-Tremblay et al., 2014 | Prospective cohort (Project Ice Storm) | N = 68 | Any time during pregnancy (depending on when the ice storm occurred) | i) Subjective distress via the Impact of Events Scale-Revised and ii) Objective hardship via the Storm32 | Subjective distress associated with increased risk of doctor-diagnosed asthma, lifetime wheeze, and lifetime use of corticosteroids at age 12  
|                       |                         |        |                | Objective distress not associated with doctor-diagnosed asthma, lifetime wheeze, or lifetime use of corticosteroids at age 12 |                                                                         |
| Chiu et al., 2012     | Prospective cohort (ACCESS*)          | N = 653 urban English- & Spanish-speaking | Throughout pregnancy (assessed at 28 ± 8 weeks gestation) | Stressful life events via the CRISYS-R* (number of domains with any negative events)             | PNMS associated with wheeze up to age 2  
<p>|                       |                         |        |                | Sub-analysis: PNMS associated with wheeze up to age 2 only in non-atopic/non-sensitized mothers       |                                                                         |
| deMarco et al., 2012  | Cross-sectional           | N = 3,854 (from a single geographic | Throughout pregnancy, retrospective recall of PNMS | Stressful life events (mourning, divorce, job loss)                                           | PNMS associated with atopic disease in children aged 3-14 (including asthma, eczema, allergic rhinitis and wheeze) |</p>
<table>
<thead>
<tr>
<th>Publication</th>
<th>Type of Study</th>
<th>Sample Details</th>
<th>PNMS Details</th>
<th>Additional Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Khashan et al., 2012</td>
<td>Population (i.e., from Swedish Medical Register)</td>
<td>N = 3,193,033 born 1973-2004 Any time during pregnancy or in the 6 months prior Bereavement of child or spouse (gathered from registry data)</td>
<td>PNMS (i.e., bereavement from loss during pregnancy only) associated with increased risk of offspring asthma hospitalization. Association remains when examining only offspring over 6 years of age.</td>
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<tr>
<td>Fang et al., 2011</td>
<td>Population (i.e., from Swedish Medical Register)</td>
<td>i) N = 449,363 born July 1, 2004-Dec. 31, 2008 (&quot;younger sample&quot;) ii) N = 514,261 born Jan. 1, 1997-Dec. 31, 2002 (&quot;older sample&quot;) Any time during pregnancy or in the 1 year prior Bereavement of a close relative</td>
<td>PNMS associated with asthma at age 1-4 in boys, especially if PNMS occurred in the 2nd trimester (younger sample) PNMS associated with asthma attacks at 7-12 years in boys (older sample)</td>
<td></td>
</tr>
<tr>
<td>Reyes et al., 2011</td>
<td>Prospective cohort</td>
<td>N = 279 urban, low SES, minority 3rd trimester 'Demoralization' (composite of: anxiety, sadness, perceived physical health, poor self-esteem, dread, confused thinking, hopelessness/helplessness, and psychophysiological symptoms)</td>
<td>PNMS associated with overall wheeze by age 5. PNMS associated with transient wheeze and with persistent wheeze PNMS not associated with total IgE levels or allergen-specific IgE levels at age 2, 3 &amp; 5</td>
<td></td>
</tr>
<tr>
<td>Sternthal et al., 2011</td>
<td>Prospective cohort (ACCESS*)</td>
<td>N = 510 urban English- &amp; Spanish-speaking Throughout pregnancy (assessed at 28 ± 8 weeks gestation) Stressful life events via the CRISYS-R** (number of domains with any negative events)</td>
<td>PNMS associated with wheeze at age 2</td>
<td></td>
</tr>
<tr>
<td>Wen et al., 2011</td>
<td>Prospective cohort</td>
<td>N = 730 3rd trimester (assessed about 1 month before due date) Stress Factor derived from SF-36** (composite of: nervousness, depression, anxiety, exhaustion, tiredness, working stress)</td>
<td>PNMS associated with atopic dermatitis at age 2</td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>Study Type</td>
<td>Sample Size at Age 6</td>
<td>Sampling Time</td>
<td>Stressful Life Events</td>
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<tr>
<td>Kozyrskyj et al., 2010</td>
<td>Prospective cohort</td>
<td>N = 2,151</td>
<td>Mid-pregnancy</td>
<td>Stressful life events (&gt; 3 stressful life events) via the Tennant and Andrews validated life-events instrument</td>
</tr>
<tr>
<td></td>
<td>(Raine study*)</td>
<td>at age 6 and N = 1,796 at age 14</td>
<td>(assessed after recruitment at 16-20 weeks gestation)</td>
<td></td>
</tr>
<tr>
<td>Cookson et al., 2009</td>
<td>Population cohort</td>
<td>N = 5,810</td>
<td>i) 2nd and ii) 3rd trimesters</td>
<td>Anxiety via Crown-Crisp Experiential Index</td>
</tr>
<tr>
<td></td>
<td>(ALSPAC*)</td>
<td>(assessed at 18 &amp; 32 weeks gestation)</td>
<td></td>
<td>PNMS not associated with atopic sensitization at age 7 (i.e., positive skin-prick test)</td>
</tr>
<tr>
<td>Sausenthaler et al., 2009</td>
<td>Prospective cohort</td>
<td>N = 2,2013</td>
<td>Throughout pregnancy</td>
<td>2 or more ‘stressors’ (9 physical, 3 psychological) derived from prenatal charts, except unwanted pregnancy – assessed by interview shortly after birth</td>
</tr>
</tbody>
</table>

*Cohort Studies: ACCESS = Asthma Coalition on Community, Environment, and Social Stress project; ALSPAC = Avon Longitudinal Study of Parents and Children; LISA = Influences of lifestyle related factors on the immune system and the development of allergies in childhood; Raine = Western Australia Pregnancy Cohort

**Questionnaires: SF-36 = Short Form Health Survey; CRISYS-R = Crisis in Family Systems – Revised

Other Abbreviations: PNMS = Prenatal maternal stress; SES = Socioeconomic status
Appendix B: Human Studies of Prenatal Stress (PNMS) and Atopic Markers in Cord Blood

<table>
<thead>
<tr>
<th>Authors</th>
<th>Study Design</th>
<th>Sample</th>
<th>Timing of PNMS</th>
<th>Type of PNMS</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peters et al., 2012</td>
<td>Prospective cohort (ACCESS)</td>
<td>N = 403 urban, low SES, minority</td>
<td>Throughout pregnancy (assessed at 29 ± 5 weeks gestation)</td>
<td>Stressful life events via the CRISYS-R (number of domains with any negative events)</td>
<td>PNMS associated with high levels of cord blood IgE in atopic mothers only</td>
</tr>
<tr>
<td>Sternthal et al., 2011</td>
<td>Prospective cohort (ACCESS)</td>
<td>N = 454 urban English- &amp; Spanish-speaking</td>
<td>Throughout pregnancy (assessed at 28 ± 8 weeks gestation)</td>
<td>Stressful life events via the CRISYS-R (number of domains with any negative events)</td>
<td>PNMS not associated with cord blood IgE levels</td>
</tr>
<tr>
<td>Mattes et al., 2009</td>
<td>Randomized control trial (of prenatal fish oil supplement)</td>
<td>N = 83</td>
<td>2nd trimester (assessed at 20 weeks gestation)</td>
<td>Depression via BDI</td>
<td>PNMS associated with cord blood lymphoproliferative and cytokine responses to allergens</td>
</tr>
<tr>
<td>Lin et al., 2004</td>
<td>Cross-sectional</td>
<td>N = 334</td>
<td>3rd trimester (one month before birth)</td>
<td>Psychosocial stress factor via SF-36 (composite of: anxiety, nervousness, exhaustion, tiredness, working stress, discouragement)</td>
<td>PNMS associated with elevated cord blood IgE levels</td>
</tr>
</tbody>
</table>

*Abbreviations: ACCESS = Asthma Coalition on Community, Environment, and Social Stress project; BDI = Beck Depression Inventory; PNMS = prenatal maternal stress SES = Socioeconomic status; SF-36 = Short Form Health Survey; CRISYS-R = Crisis in Family Systems – Revised*
Appendix C: Human Studies of Prenatal Stress (PNMS) and Offspring *in vitro* Immune Function

<table>
<thead>
<tr>
<th>Authors</th>
<th>Study Design</th>
<th>Sample</th>
<th>Timing of PNMS</th>
<th>Type of PNMS</th>
<th>Outcome</th>
</tr>
</thead>
</table>
| Veru et al., 2015 | Prospective cohort (Project Ice Storm) | N = 37                                                                  | Any time during pregnancy (depending on when the ice storm occurred) | i) Subjective distress via the Impact of Events Scale-Revised and ii) Objective hardship via the Storm32 | Objective PNMS negatively correlated with total lymphocytes and number of T helper cells at age 13  
Objective PNMS positively associated with *in vitro* stimulated TNF-α, IL-1β, IL-6, IL-4, IL-13 at age 13 (associations with TNF-α, IL-4 and IL-13 remained in regression analyses)  
Subjective PNMS not associated with cell numbers of stimulated cytokine production at age 13 |
| O’Connor et al., 2013 | Prospective                  | N = 80, N =76 for humoral responses at 2 and 6 months respectively  
N = 56, N =54 for cellular responses at 2 and 6 months respectively | 2nd and 3rd trimester (averaged)                                 | Anxiety via the Penn State Worry Questionnaire                               | PNMS associated with *in vitro* Th2 skew of T cells. Specifically decreased antibody titers at 6 months, decreased IFN-γ and increased IL-4 cell responder frequencies to antigens at 6 months (but not at 2 months)  
Sub-Analysis (n=30): PNMS associated with decreased serum IL-12 (Th1 cytokine) at 2 months (but not at 6 months), and IL-12 correlated with IFN-γ |
| Entringer et al., 2008 | Retrospective                 | N = 34 exposed to PNMS, N = 28 healthy controls                       | Any time during pregnancy (assessed retrospectively 25 ± 4 years post-birth) | Stressful life events                                                       | PNMS associated with increased *in vitro* stimulated IL-4, IL-6 and IL-10 and lower *in vitro* stimulated IFN-γ:IL-4  
PNMS not associated with lymphocyte numbers or the ratio of T helper to cytotoxic T cells at age 25 |

*Abbreviations: PNMS = prenatal maternal stress*
### Appendix D: Allergen Type and Concentrations for Maternal and Infant Skin Prick Testing

<table>
<thead>
<tr>
<th>Allergen</th>
<th>Specific Allergen Type</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fungus (mould)</strong></td>
<td>Alternaria tenuis (aka Alternaria alternata)</td>
<td>1:10 W/V</td>
</tr>
<tr>
<td><strong>Fungus</strong></td>
<td>Cladosporium sphaerospermum</td>
<td>1:10 W/V</td>
</tr>
<tr>
<td><strong>Fungus</strong></td>
<td>Aspergillus fumigatus</td>
<td>1:10 W/V</td>
</tr>
<tr>
<td><strong>Dog</strong></td>
<td>Dog epithelium</td>
<td>1:20 W/V</td>
</tr>
<tr>
<td><strong>Cat</strong></td>
<td>Cat pelt</td>
<td>Standardized, glycerinated - 10,000 BAU/mL</td>
</tr>
<tr>
<td><strong>Horse</strong></td>
<td>Horse epithelium</td>
<td>1:20 W/V</td>
</tr>
<tr>
<td><strong>Feather mix</strong></td>
<td>Chicken, Duck, Goose</td>
<td>1:20 W/V</td>
</tr>
<tr>
<td><strong>Cockroach</strong></td>
<td>American, German</td>
<td>1:20 W/V</td>
</tr>
<tr>
<td><strong>Dust mite</strong></td>
<td>Dermatophagoides farinae</td>
<td>Glycerinated – 10,000 AU/mL</td>
</tr>
<tr>
<td><strong>Dust mite</strong></td>
<td>Dermatophagoides pteronyssinus</td>
<td>Glycerinated – 10,000 AU/mL</td>
</tr>
<tr>
<td><strong>Milk</strong></td>
<td>Whole, Cow’s</td>
<td>1:10 W/V</td>
</tr>
<tr>
<td><strong>Egg</strong></td>
<td>White, Chicken</td>
<td>1:10 W/V</td>
</tr>
<tr>
<td><strong>Wheat</strong></td>
<td>Whole, Wheat Grain</td>
<td>1:10 W/V</td>
</tr>
<tr>
<td><strong>Peanut</strong></td>
<td></td>
<td>1:10 W/V</td>
</tr>
<tr>
<td><strong>Nut mix</strong></td>
<td>Brazil nut, Walnut</td>
<td>1:20 W/V</td>
</tr>
<tr>
<td><strong>Tree mix</strong></td>
<td>Alder, Ash, Beech, Birch, Elm, Hickory, Maple, Oak, Poplar, Sycamore</td>
<td>1:40 W/V</td>
</tr>
<tr>
<td><strong>Grass pollen mix</strong></td>
<td>Timothy, Orchard, June, Red Top, Sweet Vernal</td>
<td>1:40 W/V</td>
</tr>
<tr>
<td><strong>Ragweed pollen</strong></td>
<td>Tall, Short</td>
<td>1:40 W/V</td>
</tr>
<tr>
<td><strong>Weed mix</strong></td>
<td>Cocklebur, Rough Marshelder, English Plantain, Lamb’s Quarters</td>
<td>1:40 W/V</td>
</tr>
<tr>
<td><strong>Histamine</strong></td>
<td>Histatrol</td>
<td>10 mg/mL</td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td>Glycerin</td>
<td>50%</td>
</tr>
</tbody>
</table>

*W/v = weight to volume ratio, BAU = Bioequivalent Allergy Unit*
Appendix E: Minimum Detectable Concentrations, Intra- and Inter-Assay % CV for Serum Cytokines

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Minimum detectable concentration (pg/mL)</th>
<th>Intra-Assay % CV</th>
<th>Inter-Assay % CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td>0.8</td>
<td>2.3</td>
<td>6.7</td>
</tr>
<tr>
<td>IL-4</td>
<td>4.5</td>
<td>2.9</td>
<td>14.2</td>
</tr>
<tr>
<td>IL-5</td>
<td>0.5</td>
<td>2.6</td>
<td>10.8</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.9</td>
<td>2.0</td>
<td>18.3</td>
</tr>
<tr>
<td>IL-10</td>
<td>1.1</td>
<td>1.6</td>
<td>16.8</td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.7</td>
<td>2.6</td>
<td>13.0</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>0.8</td>
<td>1.6</td>
<td>12.0</td>
</tr>
</tbody>
</table>
Table #F1: Missing Demographic, Medical, and Questionnaire Data at Visit #1

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current Medication Use</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td>Psychiatric History</td>
<td>2</td>
<td>2.6</td>
</tr>
<tr>
<td>Level of Education</td>
<td>2</td>
<td>2.6</td>
</tr>
<tr>
<td>Planned Pregnancy</td>
<td>3</td>
<td>3.8</td>
</tr>
<tr>
<td>Current Use of Cigarettes</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td>Prime MD Patient Health Questionnaire (PHQ)</td>
<td>7</td>
<td>9.0</td>
</tr>
<tr>
<td>Maternal Report of Physician-Diagnosed Allergy</td>
<td>9</td>
<td>11.5</td>
</tr>
<tr>
<td>PSS</td>
<td>4</td>
<td>5.1</td>
</tr>
<tr>
<td>EPDS</td>
<td>3</td>
<td>3.8</td>
</tr>
<tr>
<td>CRISYS-R-neg</td>
<td>2</td>
<td>2.6</td>
</tr>
</tbody>
</table>

*n = 0 for maternal age, parity, current work status, country of origin, time since immigration, presence of a language barrier, and MSPSS

Table #F2: Missing Questionnaire Data at Visit #2

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>%</th>
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</thead>
<tbody>
<tr>
<td>CRISYS-R-neg</td>
<td>2</td>
<td>3.5</td>
</tr>
<tr>
<td>EPDS</td>
<td>1</td>
<td>1.8</td>
</tr>
<tr>
<td>MSPSS</td>
<td>1</td>
<td>1.8</td>
</tr>
</tbody>
</table>

*n = 0 for pregnancy-related complications, current medication use, and PSS
### Table #F3: Missing Medical and Questionnaire Data at Visit #3

<table>
<thead>
<tr>
<th>Variable</th>
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<th>%</th>
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</thead>
<tbody>
<tr>
<td>Diagnosed Allergies in the infant</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>Length of Breastfeeding</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>PSS</td>
<td>6</td>
<td>18</td>
</tr>
<tr>
<td>CRISYS-R-neg</td>
<td>6</td>
<td>18</td>
</tr>
<tr>
<td>EPDS</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>MSPSS</td>
<td>6</td>
<td>18</td>
</tr>
<tr>
<td>RS</td>
<td>10</td>
<td>29</td>
</tr>
</tbody>
</table>

*n = 0 for infant age, infant sex, and self-reported maternal and infant health*
Appendix G: Logistic Regression Using Prenatal Stress Variables at Visit #2 to Predict Infant Allergic Outcomes

Table #G1: Logistic Regression to Predict Infant Skin Prick Test Response using CRISYS-R-neg scores at visit #2

<table>
<thead>
<tr>
<th>Omnibus Test of Model Coefficients</th>
<th>$\chi^2$</th>
<th>df</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.185</td>
<td>4</td>
<td>0.527</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Model Summary</th>
<th>Nagelkerke's $R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.196</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Prediction Success</th>
<th>Overall</th>
<th>Negative SPT</th>
<th>Positive SPT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>89%</td>
<td>100%</td>
<td>25%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Predictor</th>
<th>B</th>
<th>SE</th>
<th>df</th>
<th>Exp(B)</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infant sex (female)</td>
<td>1.406</td>
<td>1.341</td>
<td>1</td>
<td>4.080</td>
<td>0.295</td>
</tr>
<tr>
<td>Infant age (months)</td>
<td>0.209</td>
<td>0.147</td>
<td>1</td>
<td>1.233</td>
<td>0.154</td>
</tr>
<tr>
<td>Maternal SPT response (positive)</td>
<td>-0.448</td>
<td>1.454</td>
<td>1</td>
<td>0.639</td>
<td>0.758</td>
</tr>
<tr>
<td>CRISYS-R-neg score at visit #2</td>
<td>-0.362</td>
<td>0.356</td>
<td>1</td>
<td>0.696</td>
<td>0.309</td>
</tr>
</tbody>
</table>
Table #G2: Logistic Regression to Predict Physician-Diagnosed Allergy in the Infant using CRISYS-R-neg scores at visit #2

<table>
<thead>
<tr>
<th>Omnibus Test of Model Coefficients</th>
<th>$\chi^2$</th>
<th>df</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.117</td>
<td>4</td>
<td>0.892</td>
</tr>
</tbody>
</table>

| Model Summary | Nagelkerke's $R^2$ | 0.082 |

<table>
<thead>
<tr>
<th>Prediction Success</th>
<th>Overall</th>
<th>Negative SPT</th>
<th>Positive SPT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>89%</td>
<td>100%</td>
<td>0%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Predictor</th>
<th>B</th>
<th>SE</th>
<th>df</th>
<th>Exp(B)</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infant sex (female)</td>
<td>1.377</td>
<td>1.434</td>
<td>1</td>
<td>3.962</td>
<td>0.337</td>
</tr>
<tr>
<td>Infant age (months)</td>
<td>0.012</td>
<td>0.178</td>
<td>1</td>
<td>1.012</td>
<td>0.947</td>
</tr>
<tr>
<td>Maternal SPT response (positive)</td>
<td>0.023</td>
<td>1.374</td>
<td>1</td>
<td>1.024</td>
<td>0.986</td>
</tr>
<tr>
<td>CRISYS-R-neg score at visit #2</td>
<td>0.015</td>
<td>0.349</td>
<td>1</td>
<td>1.015</td>
<td>0.965</td>
</tr>
</tbody>
</table>
### Table #G3: Logistic Regression to Predict Infant Skin Prick Test Response using PSS scores at visit #2

<table>
<thead>
<tr>
<th>Omnibus Test of Model Coefficients</th>
<th>$\chi^2$</th>
<th>df</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4.348</td>
<td>4</td>
<td>0.361</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Model Summary</th>
<th>Nagelkerke's $R^2$</th>
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</thead>
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<td>0.257</td>
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<table>
<thead>
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<th>Overall</th>
<th>Negative SPT</th>
<th>Positive SPT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>86%</td>
<td>100%</td>
<td>0%</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Predictor</th>
<th>B</th>
<th>SE</th>
<th>df</th>
<th>Exp(B)</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infant sex (female)</td>
<td>1.722</td>
<td>1.472</td>
<td>1</td>
<td>5.595</td>
<td>0.242</td>
</tr>
<tr>
<td>Infant age (months)</td>
<td>0.197</td>
<td>0.148</td>
<td>1</td>
<td>1.217</td>
<td>0.184</td>
</tr>
<tr>
<td>Maternal SPT response (positive)</td>
<td>-0.420</td>
<td>1.562</td>
<td>1</td>
<td>0.657</td>
<td>0.788</td>
</tr>
<tr>
<td>PSS score at visit #2</td>
<td>-0.198</td>
<td>0.140</td>
<td>1</td>
<td>0.820</td>
<td>0.156</td>
</tr>
</tbody>
</table>
Table #G4: Logistic Regression to Predict Physician-Diagnosed Allergy in the Infant using PSS scores at visit #2

<table>
<thead>
<tr>
<th>Omnibus Test of Model Coefficients</th>
<th>$\chi^2$</th>
<th>df</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.91</td>
<td>4</td>
<td>0.573</td>
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</tbody>
</table>

| Model Summary | Nagelkerke’s $R^2$ | 0.203 |

<table>
<thead>
<tr>
<th>Prediction Success</th>
<th>Overall</th>
<th>Negative SPT</th>
<th>Positive SPT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>89%</td>
<td>100%</td>
<td>0%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Predictor</th>
<th>B</th>
<th>SE</th>
<th>df</th>
<th>Exp(B)</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infant sex (female)</td>
<td>1.387</td>
<td>1.610</td>
<td>1</td>
<td>4.005</td>
<td>0.389</td>
</tr>
<tr>
<td>Infant age (months)</td>
<td>0.090</td>
<td>0.195</td>
<td>1</td>
<td>1.095</td>
<td>0.643</td>
</tr>
<tr>
<td>Maternal SPT response (positive)</td>
<td>0.078</td>
<td>1.413</td>
<td>1</td>
<td>1.082</td>
<td>0.956</td>
</tr>
<tr>
<td>PSS score at visit #2</td>
<td>0.203</td>
<td>0.162</td>
<td>1</td>
<td>1.225</td>
<td>0.210</td>
</tr>
</tbody>
</table>
Appendix H: Comparisons of Participants Who Did and Did Not Persist in the Study to Completion

Table H1: Comparison of Participants Who Did and Did Not Persist in the Study To Completion (t-tests)

<table>
<thead>
<tr>
<th>Completed Study?</th>
<th>n</th>
<th>Mean ± SD</th>
<th>t</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>44</td>
<td>29.8 ± 5.7</td>
<td>0.38</td>
<td>76</td>
<td>0.706</td>
</tr>
<tr>
<td>Yes</td>
<td>34</td>
<td>30.2 ± 4.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time since immigration (months)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>44</td>
<td>50.5 ± 48.5</td>
<td>1.54</td>
<td>50.34</td>
<td>0.129</td>
</tr>
<tr>
<td>Yes</td>
<td>34</td>
<td>75.1 ± 82.3</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>EPDS at visit #1</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>43</td>
<td>6.7 ± 5.3</td>
<td>0.18</td>
<td>70.87</td>
<td>0.861</td>
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<tr>
<td>Yes</td>
<td>32</td>
<td>6.5 ± 3.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PSS at visit #1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>42</td>
<td>14.6 ± 6.5</td>
<td>0.51</td>
<td>72</td>
<td>0.613</td>
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<tr>
<td>Yes</td>
<td>32</td>
<td>13.8 ± 6.4</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>CRISYS-R-neg at visit #1</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>43</td>
<td>2.3 ± 2.2</td>
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<td>74</td>
<td>0.623</td>
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<tr>
<td>Yes</td>
<td>33</td>
<td>2.6 ± 2.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSPSS at visit #1</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
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<td>70.0 ± 8.8</td>
<td>0.92</td>
<td>76</td>
<td>0.359</td>
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<tr>
<td>Yes</td>
<td>34</td>
<td>71.9 ± 10.6</td>
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<td></td>
</tr>
<tr>
<td>EPDS at visit #2</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>27</td>
<td>7.6 ± 5.1</td>
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$p < 0.10$
Table H2: Comparison of Participants Who Did and Did Not Persist in the Study To Completion (chi-square tests)

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§Fisher's exact test, two-sided; *p < 0.05