STRESS, DEPRESSION, AND INFLAMMATORY IMMUNE RESPONSES DURING PREGNANCY

DISSERTATION

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By

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ABSTRACT

Psychosocial stress and depressive symptoms predict increased risk of negative perinatal outcomes including preterm delivery and gestational hypertension. Inflammation is a key potential mechanism by which stress and depressive symptoms may influence such outcomes. The current study examined associations among stress, depressive symptoms, and inflammation during pregnancy. It was hypothesized that women reporting greater stress and/or depressive symptoms would exhibit higher serum levels of the proinflammatory cytokine interleukin-6 (IL-6) and exhibit an exaggerated IL-6 response upon exposure to an antigen challenge of influenza vaccination.

Psychosocial factors and serum levels of interleukin-6 were assessed prior to vaccination (n=60) at 1-2 weeks post-vaccination (n=37) in a sample of pregnant women. Of the 60 women who completed the baseline session, the majority were African-American (57%), had completed high school or less education (82%), and reported a total annual family income of less than $15,000 per year (63%). Psychosocial measures included the 4-item Perceived Stress Scale (PSS) and the Center for Epidemiological Studies Depression Scale (CES-D). Results demonstrated that 31 women (52%) scored at or above a clinical cut-off for depressive symptoms. Serum levels of IL-6 were determined using high sensitivity immunoassays. Regression analyses indicated that after controlling for body
mass index (BMI) prior to pregnancy, higher scores on the CES-D were predictive of higher levels of IL-6 at baseline ($\beta=.23, p=.05$). There was no significant change in IL-6 from baseline to the post-vaccination timepoint ($p > .05$). Moreover, neither stress nor depressive symptoms predicted IL-6 responses to vaccination ($ps > .05$). In sum, the current results indicate that depressive symptoms predict higher levels of maternal serum IL-6 during pregnancy. These data are consistent with the contention that depressive symptoms may contribute to negative perinatal outcomes via inflammatory pathways.
Dedicated to

my mother, Mary Alice

who I see in myself more everyday,

my father, James

who has spoiled me rotten,

and my husband, Corey

who continues the tradition.
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CHAPTER 1
INTRODUCTION

Psychosocial stress and negative affect, measured in various ways, are predictive of perinatal outcomes including preterm birth and low birth weight (Copper et al., 1996; Dole et al., 2003; Lobel, DeVincent, Kaminer, & Meyer, 2000; Nordentoft et al., 1996; Wadhwa, Sandman, Porto, Dunkel-Schetter, & Garite, 1993), gestational hypertension (Kurki, Hiilesmaa, Raitasalo, Mattila, & Ylikorkala, 2000; Paarlberg, Vingerhoets, Passchier, Dekker, & van Geijin, 1995), and mental and physical characteristics of offspring (McEwen & Seeman, 1999; Weinstock, 2001). These associations generally remain after controlling for possible behavioral and demographic explanations including health behaviors and socioeconomic status. This suggests a role for more direct physiological pathways. However, limited research has attempted to identify physiological mechanisms linking psychosocial stress and perinatal outcomes.

A key potential mechanism underlying the association between stress and perinatal outcomes is inflammation. Proinflammatory cytokines, including interleukin (IL)-6, IL-1, and tumor necrosis factor (TNF), are proteins with many functions that are released by multiple cells in the body including fat cells, endothelial cells, and immune cells (Vilcek, 2003). Most relevant to the current review, proinflammatory cytokines are
released by immune cells when a threat such as a virus or wound is detected. One function of these cytokines is to cause inflammation. An inflammatory response involves increased vascular permeability, the recruitment of key proteins and immune cells to the affected area, and is characterized by redness, swelling, and pain (Rabin, 1999). Chronic inflammation is believed to contribute to the development of a number of serious health conditions including cardiovascular diseases, arthritis, diabetes, inflammatory bowel disease, periodontal disease, certain cancers, and age-related functional decline (Black, 2002; Bruunsgaard, Pedersen, & Pedersen, 2001; Ershler & Keller, 2000; Hamerman, Berman, Albers, Brown, & Silver, 1999; Ishihara & Hirano, 2002).

Research linking psychological factors and inflammation during pregnancy is limited. However, in non-pregnant populations, stress, anxiety, and depressive symptoms have been associated with higher levels of circulating inflammatory cytokines as well as greater inflammatory responses to psychological stressors and biological challenges (Irwin, 2002; Lutgendorf et al., 1999; Maes et al., 1997; Miller, 1998; Musselman et al., 2001; Schiepers, Wichers, & Maes, 2005; Zorrilla et al., 2001). The degree to which these findings generalize to pregnant populations is not known.

Understanding the effect of psychosocial factors on inflammatory processes during pregnancy is critical because successful pregnancy is characterized by suppression of certain inflammatory aspects of immune function (Wilder, 1998). This shift presumably helps to prevent rejection of the fetus by the maternal immune system (Blackburn & Loper, 1992; Stables, 1999). The most thoroughly studied proinflammatory cytokine in relation to perinatal outcomes is interleukin-6 (IL-6). As will be reviewed,
high levels of IL-6 are causally associated with the development of gestational hypertension, preterm birth, and impaired offspring development (Conrad, Miles, & Benyo, 1998; Granger, 2004; Knackstedt, Hamelmann, & Arck, 2005; Lockwood, 1999).

The current study will examine the association between self-reported stress, depressive symptoms, and IL-6 in pregnant women at baseline and in response to influenza vaccination. To provide a context and rationale for the design and specific hypotheses of the current investigation, literature will be reviewed linking: 1) stress/depression and perinatal outcomes, 2) inflammation and perinatal outcomes, and 3) stress/depression and inflammation. Although conceptually distinct, stress and depression are similar in that they involve negative mood, activation of the hypothalamic-pituitary-adrenal (HPA) axis, and associated negative health outcomes (Anisman & Merali, 2003; Connor & Leonard, 1998). Therefore, literature regarding these two constructs will be reviewed together.

**Stress/Depression and Perinatal Outcomes**

Pregnancy is often a time of joy and positive anticipation. However, it is also period of great change and preparation that can certainly be perceived as stressful (Roesch, Dunkel-Schetter, Woo, & Hobel, 2004). Moreover, women differ in their ability to cope with the life changes associated with pregnancy (Lederman, 1984). In addition to the general experience of stress, clinical symptoms of depression are common. It is estimated that 9-10% of women meet criteria for major depression during pregnancy (Gotlib, Whiffen, Mount, Milne, & Cordy, 1989; O'Hara, Neunaber, & Zekoski, 1984). In
fact, depression may be more common during pregnancy than during the postpartum period (Evans, Heron, Francomb, Oke, & Golding, 2001). These estimates are conservative; it is likely that depression is under-diagnosed during pregnancy because symptoms such as sleep disturbance, appetite change, and fatigue may be incorrectly attributed to pregnancy rather than depression (Cott & Wisner, 2003). Indeed, in a recent study of nearly 3,500 pregnant women, 20% scored above a clinical cut-off for significant depressive symptomatology (Marcus, Flynn, Blow, & Barry, 2003).

Due to ethical considerations, much of the literature exploring the effects of stress during pregnancy has utilized animal models. Animal models provide excellent experimental data that allow for causal interpretation. However, subjective affect cannot be measured, limiting the scope of assessment to objective behavioral and physiological measures. In contrast, research of stress and pregnancy with humans is non-experimental in design, limiting the ability to determine causality. Despite limitations, the strengths of human and animal designs complement each other; together these models provide strong evidence that psychological stress affects perinatal outcomes.

Gestational Hypertension. Hypertensive disorders of pregnancy, including gestational hypertension and preeclampsia, are responsible for 12-15% of pregnancy-related deaths worldwide (Page, 2002, World health report, 2005) and are a leading cause of premature delivery (Pschirrer & Monga, 2000). Affecting 5-10% of all pregnancies in the United States (Granger, Alexander, Bennett, & Khalil, 2002), preeclampsia is a particularly serious condition marked by hypertension as well as edema and high levels of protein in the urine (proteinuria) which indicates kidney dysfunction (Granger, 2004).
There is no treatment for preeclampsia; bedrest is typically prescribed. Because symptoms are relieved only after delivery, labor is often induced early to prevent serious consequences to maternal and fetal health (World health report, 2005).

The origins of hypertensive disorders during pregnancy are not clearly delineated, but multiple factors are implicated (Page, 2002). Stress, anxiety, and depression appear to contribute to risk. For example, in pregnant rats, cold stimulation of the paws administered continuously for two weeks induced symptoms similar to preeclampsia (Kanayama, Tsujimura, She, Maehara, & Terao, 1997; Khatun et al., 1999), suggesting that excessive stimulation of the sympathetic nervous system can contribute to the development of the disorder (Arck, 2001).

Human studies demonstrate the link between psychological factors and preeclampsia. In a sample of 623 Finnish women, those who reported higher levels of anxiety or depressive symptoms early in pregnancy, prior to the development of preeclampsia, were 2-3 times more likely to develop the disorder than their less anxious or depressed counterparts (Kurki et al., 2000). In a retrospective study of 717 women, those reporting greater work stress in the first trimester experienced increased risk of preeclampsia in the third trimester (Landbergis, 1996). In particular, having a job characterized by both low decisional-latitude and high job pressure was associated with greater risk (Landbergis, 1996). While not found in all studies (Nisell, Larsson, & Wager, 1989), evidence also supports a link between self-reported stressful life events and likelihood of preeclampsia (Hetzel, Bruer, & Poidevin, 1961). Similar results were found using objectively determined stress; among 5,804 pregnant Israeli women living in a war
environment, those classified as living in higher risk areas had higher blood pressure than women living in lower risk areas (Rofe & Goldberg, 1983). Given accumulating evidence linking stress, depressive symptoms, and hypertensive disorders of pregnancy, exploration of potential physiological mechanisms underlying these relationships is warranted.

Preterm Birth and Low Birth Weight. In industrialized countries, the leading causes of infant mortality are preterm birth and low birth weight (Kramer et al., 2001). In the United States, approximately 12% of births occur preterm, the majority with no known cause (Berkowitz & Papiernik, 1993; Kramer, 1987). Gestational age at delivery and birth weight are related but somewhat independent; approximately 60% of low birth weight infants are born preterm (Mattison, Damus, Fiore, Petrini, & Alter, 2001). Both preterm birth and low birth weight are associated with serious health complications in infants, substantially increased risk of infant death, and significant financial burden (Mattison et al., 2001). Infants who are both preterm and of low birth weight evidence the greatest complications (Mattison et al., 2001).

After accounting for the role of preeclampsia, as well as other known risk factors for preterm birth, an association between stress/depression and preterm birth remains (for reviews see(Bonari et al., 2004; Paarlberg et al., 1995). This association is quite consistent across studies, a number of which are prospective in design (Copper et al., 1996; Dole et al., 2003; Hedegaard, Brink Henriksen, Sabroe, & Secher, 1993; Levi, Lundberg, Hanson, & Frankenhauser, 1989; Lobel, Dunkel-Schetter, & Scrimshaw, 1992; Nordentoft et al., 1996; Omer, Friedlander, Palti, & Shekel, 1986; Pritchard, 1994;
Steer, Scholl, Hediger, & Fischer, 1992; Wadhwa et al., 1993). Although results are less consistent, maternal stress and depression have also predicted low birth weight (< 2500 grams; Paarlberg et al., 1995). For example, in a population-based study of 2,378 mothers, the risk of having a baby with very low birth weight was 1.5 times greater among women reporting that they “almost always” felt stressed during their pregnancies after controlling for other known predictors of low birth weight including maternal tobacco use, maternal age, and medical problems (Sable & Wilkinson, 2000).

The effects of stress may be most evident in at-risk populations. In a study of 130 women of low socioeconomic status, women who reported high levels of stress who were also classified as high medical risk had substantially increased likelihood of preterm delivery compared to those experiencing either factor (high stress or high medical risk) alone (Lobel et al., 1992). In addition, the link between stress and preterm birth may be partially mediated by increased susceptibility to infections. In a multisite study of 454 pregnant women, those in the top two quartiles of reported stress had approximately two-fold greater risk of bacterial vaginosis (BV) than did women in the bottom quartile after controlling for sociodemographic variables related to BV risk including race, vaginal douching, and number of lifetime sexual partners (Culhane et al., 2001).

The experience of psychosocial stress is affected by many factors, including gender, race, socioeconomic status, and social relationships. In particular, the chronic stress of racism has been proposed as a potential cause of substantially higher rates of preterm birth and low birth weight babies seen in African-American versus European-American populations (Collins, David, Handler, Wall, & Andes, 2004; Dole et al., 2004;
Furthermore, only women can become pregnant, and stress unique to the female gender role may contribute to birth outcomes. For example, perception of dissatisfaction with one’s household role predicts low birth weight (Pritchard, 1994). In addition, women are more likely than men to be employed in jobs marked by low personal control, which is linked to a number of negative health outcomes (Landsbergis & Hatch, 1996).

Emphasizing the importance of conceptualizing pregnancy itself as a potential stressor, some research has found pregnancy-specific anxiety to be an even better predictor of gestational length than is general psychosocial stress (Rini, Dunkel-Schetter, Wadhwa, & Sandman, 1997; Wadhwa et al., 1993). For example, in a prospective study of 90 women, each unit increase in pregnancy-specific anxiety predicted a decrease of 3-days in gestational age at delivery after controlling for age, education, income, marital status, and medical risk (Wadhwa et al., 1993).

Offspring Characteristics. In addition to maternal health and birth outcomes, maternal stress can affect various offspring characteristics, including cognitive development, behavior, and physiology. Both the nervous and endocrine systems are key to the regulation of physiological stress responses. Because these systems are particularly malleable during fetal development and early childhood, exposure to stress in early life may have substantial and lasting effects on physiology (Coe, Kramer, Kirschbaum, Netter, & Fuchs, 2002). In fact, early “programming” processes that occur in response to the environment are believed to set the course for physiological responses through adolescence and adulthood (Caldji et al., 1998; Gunnar & Donzella, 2002; Welberg &
Seckl, 2001), affecting vulnerability to mental and physical health conditions throughout life (Allister, Lester, Carr, & Liu, 2001; Bonari et al., 2004; Lucas, Fewtrell, & Cole, 1999).

At least 14 independent prospective studies have found an association between maternal anxiety, stress, or depression, and cognitive, emotional, or behavioral outcomes in offspring from fetal development through adolescence (for a review see Van de Bergh, Mulder, Mennes, & Glover, 2005). In a study of 10 depressed women and 10 non-depressed controls, the depressed women had fetuses with higher resting heart rates and their fetuses exhibited greater heart rate responses when a mild vibrating sensation was applied to the mother’s stomach (Allister et al., 2001). Similarly, in a study of 17 women in the third trimester of pregnancy, fetuses of mothers with high trait anxiety had greater heart rate reactivity when their mothers were exposed to acute psychological stress (Monk, Myers, Sloan, Ellman, & Fifer, 2003).

In terms of neonate characteristics, of 81 newborns from healthy pregnancies, offspring of women who reported higher levels of stress during pregnancy exhibited poorer regulation of attention and greater irritability at 3-5 days old (Rieger et al., 2004). Similarly, in a study of 170 healthy infants, maternal stress and anxiety during pregnancy predicted poorer motor and mental development at 3 and 8 months of age (Huizink, Robles de Medina, Mulder, Visser, & Buitelaar, 2002). In a prospective study of 6,493 women, prenatal anxiety predicted greater behavioral problems and more difficulty regulating emotions in 4-year old boys and girls (O'Connor, Heron, Golding, & Glover, 2003). In a prospective study of 72 children, greater maternal antenatal anxiety predicted
greater symptoms of attention-deficit hyperactivity disorder and externalizing problems in 8-9 year olds (Van de Bergh & Marcoen, 2004a). In the same children, antenatal anxiety predicted greater impulsive behavior and lower scores on intelligence tests at 14 to 15 years of age (Van de Bergh et al., 2004b). Importantly, these relationships remained after controlling for key potential confounds including maternal mood after pregnancy, education level, income, smoking, gender of the child, gestational age at delivery, and birth weight.

Of particular relevance to the current review are effects of prenatal stress on offspring immune function. Findings in this area are complex; outcomes vary based on the timing and type of stressor, as well as gender of the offspring (Shanks & Lightman, 2001). However, animal models provide evidence of the powerful role of early life experiences on immune function (Coe et al., 2002; Coe & Lubach, 2005; Weinstock, 2005). In monkeys as well as rats, offspring of mothers who were repeatedly stressed during their pregnancies experienced decrements in immune function compared to offspring of undisturbed pregnancies. Specifically, their lymphocytes mounted less robust proliferative responses when exposed to an antigens in vivo or in vitro (Coe et al., 2002; Kay, Tarcic, Poltyrev, & Weinstock, 1998; Reyes & Coe, 1997). The ability of lymphocytes to proliferate, or copy themselves when activated, is key to an effective adaptive immune response.

Maternal stress may also influence offspring immune function by affecting maternal antibody production and transfer of antibodies from the mother to the fetus. In studies of non-pregnant populations, stress predicted antibody development in response
to vaccinations including influenza (Kiecolt-Glaser, Glaser, Gravenstein, Malarkey, & Sheridan, 1996) and pneumococcal pneumonia (Glaser, Sheridan, Malarkey, MacCallum, & Kiecolt-Glaser, 2000). This is important because immune function of the developing fetus and neonate is largely acquired passively from the mother (Hanson et al., 2003; Padgett, 2004). In late gestation, antibodies pass across the placenta to the developing fetus. After birth, antibodies are delivered to the neonate through breast milk. Notably, in a study with primates, repeated exposure to stress during pregnancy affected the transfer of antibodies across the placenta, although the direction of effects depended on the sex of the offspring (Coe & Crispen, 2000). These data provide another pathway by which maternal stress may impact offspring health.

Maternal stress can also alter offspring health by affecting rates of preterm birth and fetal weight. In part because maternal antibodies are transferred to the fetus primarily in the final weeks of pregnancy, infants born prematurely are likely to have significantly impaired immune function, putting them at greater risk for infection (Ballow, Cates, Rowe, Goetz, & Desbonnet, 1986). Furthermore, low birth weight has been associated with poorer antibody response to vaccination in adolescence (McDade, Beck, Kuzawa, & Adair, 2001), higher cortisol responses to acute psychosocial stress in adulthood (Wust, Entringer, Federenko, Schlotz, & Hellhammer, 2005), and increased risk of cardiovascular and metabolic disorders including diabetes later in life (Lawlor, Ronalds, Clark, Davey Smith, & Leon, 2005; Rich-Edwards et al., 1999).

An important question that remains largely unanswered is whether there is a critical period of pregnancy during which stress may be most damaging. The majority of
studies of people have assessed stress at only one timepoint, typically in the second or third trimester of pregnancy (Dunkel-Schetter, 1998). However, animal studies demonstrate that the same stressor can have different implications depending on the timing of administration. For example, in monkeys, exposure to dexamethasone (a synthetic glucocorticoid) or stress in early pregnancy resulted in increased cellular immune responses in adult offspring, while similar exposure during mid- to late pregnancy predicted decreased cellular immune responses (Coe, Lubach, & Karaszewski, 1999). Furthermore, the effects of stressor timing depend on the outcome of interest. For example, maternal transfer of antibodies to the fetus occurs mostly in the final weeks of pregnancy. Accordingly, stress during the final weeks of pregnancy most consistently affects neonate antibody levels (Coe et al., 2000). Finally, regardless of timing, it is unlikely that a single stressor will have lasting impact unless the stressor is extreme; effects in animal studies have been seen most consistently in conditions of repeated or chronic stress (Coe et al., 2000; Coe & Lubach, 2003).

**Inflammation and Perinatal Outcomes**

Despite the abundance of evidence linking maternal stress and depressive symptoms to negative perinatal outcomes, physiological mediators explaining these associations have only begun to be identified. A key potential mechanism is inflammation. As will be reviewed, inflammatory markers, particularly IL-6 and C-reactive protein (CRP), are strongly predictive of varied and important health outcomes in non-pregnant populations. Further, evidence supports the idea that successful
pregnancy is associated with a bias towards antibody-mediated immunity and away from cell-mediated inflammatory responses (Raghupathy, 1997; Wegmann, Line, Guilbert, & Mosmann, 1993).

**Cytokines as markers of inflammation.** Cytokines are proteins produced by cells of the innate and adaptive immune system as well as several other non-lymphoid cells in the body, such as adipocytes (fat cells; (Vilcek, 2003). Cytokines can be classified as pro- and antiinflammatory; however, some cytokines demonstrate both pro and anti-inflammatory characteristics. Proinflammatory cytokines promote inflammation which is characterized by the movement of lymphocytes to affected areas, increased vascular permeability, and symptoms of swelling, redness, pain, and fever (Parham, 2005). In contrast, antiinflammatory cytokines limit the immune response, in part by inhibiting the production of proinflammatory cytokines (Parham, 2005).

When considering the effects of cytokines, several characteristics are key. Notably, most cytokines are made by multiple cell types, have many functions, and can exert actions via various target cells (Vilcek, 2003). Furthermore, different cytokines, even ones that are structurally very dissimilar, can elicit similar effects (Vilcek, 2003). In addition, at any given time, a cell is typically exposed to many cytokines simultaneously and each may stimulate or inhibit the production and release of other cytokines. Therefore, the final effect of a cytokine depends on a cascade of events as well as the presence of other proteins and hormones (Kishimoto, 2003; Vilcek, 2003).

Immune responses are frequently described in terms of the ratio of T-helper-1 (Th1) or T-helper-2 (Th2) cytokines produced. Key to the adaptive immune response,
these T-cell subtypes are associated with different functional properties and produce different cytokines. Specifically, Th1 cells produce cytokines typically considered proinflammatory, including tumor necrosis factor (TNF)-alpha, IL-12, and interferon-γ (Elenkov, 2004; Mosmann & Sad, 1996; Wilder, 1998). These cytokines stimulate cellular (cell-mediated) immune responses marked by macrophage activation, inflammation, and the production of opsonizing antibodies that enhance the phagocytosis of pathogens (Parham, 2005). In contrast, Th2 cells produce antiinflammatory cytokines including IL-4, IL-5, IL-10, and IL-13 (Mosmann et al., 1996; Wilder, 1998). These cytokines promote humoral (antibody-mediated) immune responses characterized by B-cell differentiation and the production of neutralizing antibodies (Parham, 2005).

Because Th1 and Th2 cytokines tend to inhibit the production of each other, the relative balance of these cytokines is of interest. The immune system generally exhibits a tendency towards greater Th1 responding (Mosmann et al., 1996). However, this balance shifts in favor of Th2 responding in healthy pregnancy (Wilder, 1998). Specifically, successful pregnancy is associated with decreased inflammatory Th1 activity and maintained or increased antiinflammatory Th2 activity (Raghupathy, 1997; Wegmann et al., 1993; Wilder, 1998). It is presumed that suppression of inflammatory immune responses helps to prevent the rejection of the fetus which can be thought of as an allograft, or foreign tissue of the same species (Blackburn et al., 1992; Stables, 1999). Indeed, lack of a shift towards Th2 activity is harmful to the developing fetus. For example, in mice, Th1 cytokines impair implantation and trophoblast proliferation and
induce fetal resorption (Chaouat et al., 1990; Krishnan, Guilbert, Wegmann, Belosevic, & Mosmann, 1996).

Corresponding to the suppression of certain inflammatory immune responses during pregnancy, temporary improvement or remission is often seen in autoimmune disorders including inflammatory arthritis (Straub, Buttgereit, & Cutolo, 2005) and multiple sclerosis (Confavreux, Hutchinson, Hours, Cortinovis-Tourniaire, & Moreau, 1998; Langer-Gould, Garren, Slansky, Ruiz, & Steinman, 2002). However, decreases in cell-mediated immune function seen during pregnancy may also result in increased susceptibility to infection, including colds and influenza viruses (Stables, 1999).

**Interleukin-6 (IL-6).** A cytokine of much interest in the context of pregnancy is IL-6. This cytokine is classified as a Th2 cytokine; these are typically considered antiinflammatory. However, IL-6 has pro- and antiinflammatory properties (Kishimoto, 2003). In addition, IL-6 is produced by many cells in the body in addition to Th2 cells. For example, IL-6 is produced by macrophages, B-cells, fibroblasts, endothelial cells, and adipocytes (Le & Vilcek, 1989). Among other functions, IL-6 encourages lymphocyte proliferation and activation as well as antibody production (Le et al., 1989). IL-6 also stimulates the release of acute phase proteins, including as C-reactive protein (CRP) from the liver; activation of the acute phase response is key to the innate immune defense against acute infection (Kishimoto, 2003).

Outside of pregnancy, the predictive value of both IL-6 and CRP for health outcomes has received great attention. In fact, CRP is emerging as an independent risk factor for heart disease because it has demonstrated predictive value beyond known risk factors.
factors including cholesterol levels (Black, 2002; Ershler et al., 2000; Ishihara et al., 2002; Kiechl et al., 2001; Papanicolaou, Wilder, Manolagas, & Chrousos, 1998; Ridker, Cushman, Stampfer, Tracy, & Hennekens, 1997). In addition, IL-6 and CRP prospectively predicted development of type 2 diabetes in healthy women over a 4-year follow-up period in a large, population-based study (Pradhan, 2001). IL-6 and/or CRP levels have also been used to predict future functional disability, osteoporosis, and arthritis in older samples (Ferrucci et al., 1999).

Notably, high levels of IL-6 during pregnancy are predictive of infection as well as negative perinatal outcomes. Specifically, elevated levels of IL-6 measured in either amniotic fluid (R. Romero et al., 1993a) or maternal blood (Hatzidaki et al., 2005; Murtha et al., 1996) are reliable markers of neonatal infection. As will be reviewed in greater detail, high levels of IL-6 have predicted preterm birth in both idiopathic and infection-related cases (Dizon-Townson, 2001). Also to be reviewed, high levels of IL-6 in maternal circulation as well as cord blood are predictive of serious mental health problems in offspring (Yoon, Park, & Chaiworapongsa, 2003). Further, data from animals suggests that IL-6 responses to antigen exposure are reduced during normal pregnancy; pregnant rats injected with lipopolysaccaride (LPS) showed significant increases in the proinflammatory cytokine TNF-α, but not IL-6. In comparison, non-pregnant rats demonstrated increases in both of these markers (Fofie, Fewell, & Moore, 2004). Therefore, although an overall shift towards Th2 responding is seen in healthy pregnancy, high levels of IL-6 have been associated with negative perinatal outcomes.
Because the proinflammatory properties of IL-6 are key in the context of pregnancy, IL-6 will be referred to as a proinflammatory cytokine throughout.

**Inflammation and Gestational Hypertension.** Gestational hypertension and preeclampsia are conditions characterized by high levels of circulating inflammatory markers, including IL-6 (Conrad & Benyo, 1997; Conrad et al., 1998; Freeman et al., 2004; Granger, 2004; Granger, Alexander, Llinas, Bennett, & Khalil, 2001). In contrast, significantly higher levels of IL-10, a powerful anti-inflammatory cytokine, have been reported in control women compared to women with preeclampsia (Rein et al., 2003).

In addition to inflammation, placental dysfunction, abnormal lipid metabolism, and endothelial dysfunction all characterize preeclampsia (Page, 2002). Because the disorder affects multiple body systems, the degree to which noted abnormalities are markers versus causal mechanisms of preeclampsia is difficult to determine. However, evidence suggests that inflammatory immune responses are key to the initial development of the disorder. For one, many features of preeclampsia, including impaired lipid metabolism and endothelial dysfunction, can be induced by proinflammatory cytokines (Page, 2002). In addition, the clinical severity of preeclampsia has been associated with the degree of dysregulation seen in cytokine function (Madazli, Aydin, Ocak, Tolun, & Tolun, 2003).

Previous pregnancy reduces the risk of developing preeclampsia, suggesting that exaggerated inflammatory immune responses to paternal antigens may play a role in the development of the disorder. Notably, women who are pregnant for the first time (primigravid) have been estimated to be 6-8 times more likely to develop preeclampsia.
than other women (Surrat, 1993). The protective effects of previous pregnancy appear to stem from prior exposure to paternal antigens; data indicate that women who become pregnant by a different man for the first time experience the same risk of preeclampsia as primigravid women (Robillard, Dekker, & Hulsey, 1999). Furthermore, data indicate that prior exposure to paternal antigens via sperm (Koelman et al., 2000) or from previous pregnancy ending in abortion or miscarriage may result in some reduction in risk (Dekker, Robillard, & Hulsey, 1998). In sum, evidence suggests that inflammation, frequently in response to paternal antigens, plays a causal role in the development of preeclampsia.

**Inflammation and Preterm Birth.** Elevated levels of circulating proinflammatory cytokines have been associated with greater risk of pre-term delivery (Dizon-Townson, 2001). In particular, IL-6 has been linked to preterm delivery in multiple studies (Romero, Avila, Santhanam, & Sehgal, 1990; Romero et al., 1993a; Romero et al., 1993b). Inflammatory immune responses can cause preterm labor by at least three pathways, including triggering preterm contractions, encouraging cervical ripening, and causing rupture of the membranes (Hagberg, Mallard, & Jacobsson, 2005).

Approximately 10-30% of preterm labor is caused by intrauterine infection (Gomez, Ghezzi, Romero, & al., 1995); in turn, infection is believed to cause preterm delivery by stimulating inflammatory immune responses (Dudley, 1999). This suggests that women who demonstrate greater inflammatory responses to infection during pregnancy may be more likely to experience preterm birth. Furthermore, an association between high IL-6 and risk for preterm labor has also been reported in cases where there
is no detectable infection (Farina & Winkelman, 2005; Hillier et al., 1993). This suggests factors other than infection, such as stress, may stimulate inflammation to a magnitude sufficient to affect preterm birth rates (Coussons-Read, Okun, Schmitt, & Giese, 2005; Coussons-Read, Okun, & Simms, 2003).

**Inflammation and Offspring Health.** Multiple physiological pathways involving the nervous, endocrine, and immune systems are posited to mediate the relationship between maternal stress and offspring characteristics. Although the relative influence of different factors depends on the specific outcome of interest, inflammation appears to be one key pathway.

Animal models provide evidence that inflammation during pregnancy affects offspring health. In mice, maternal exposure to an endotoxin resulted in increased placental vascular resistance and cardiac dysfunction in developing fetuses (Rounioja et al., 2005). In rats, peripheral injection of IL-6 to pregnant females led to both hypertension and prolonged hormonal responses to acute stress in adult offspring (Samuelsson et al., 2004). Similarly, adult offspring of rats exposed to an endotoxic challenge had increased basal corticosterone levels as well as increased adrenocorticotropic hormone (ACTH) and corticosterone responses to acute stress (Reul et al., 1994). These data suggest that inflammation of sufficient magnitude during pregnancy can have lasting effects on offspring physiology.

In humans, the offspring characteristics that are perhaps most widely studied in the context of maternal inflammation are the neurodevelopmental disorders of cerebral palsy and schizophrenia. Offspring of women who experience infections during
pregnancy have an increased risk for both disorders (Dammann & Leviton, 1997; Yoon et al., 2003). For example, intrauterine infections and resulting inflammation are believed to account for 12% of cases of spastic cerebral palsy (Schendel, Schuchat, & Thorsen, 2002). Indicative of the role of inflammation in this association, in a sample of 172 neonates, higher levels of IL-6 in cord blood predicted occurrence of brain lesions, a key risk factor for the development of cerebral palsy (Yoon et al., 1996). Similarly, elevated circulating levels of maternal TNF-α as well as greater reporting of maternal infection during the third trimester of pregnancy were found in 27 adult offspring with schizophrenia as compared to 50 matched controls (Buka et al., 2001).

Being non-experimental in design, studies with humans cannot determine the degree to which effects of infection on the brain of the developing fetus are attributable to infection itself versus maternal inflammatory immune responses to the infection (Gayle et al., 2004). However, animal studies indicate that inflammation is a major factor. For example, stimulation of the maternal immune system by injection of LPS affected myelination in the developing rodent brain (Cai, Pan, Pang, Evans, & Rohodes, 2000) as well as the number and size of specific cells in the hippocampus of offspring (Golan, Lev, Hallak, Sorokin, & Huleihel, 2005). These effects, in turn, were associated with impairments in learning and memory (Golan et al., 2005). Also in rodents, stimulation of the immune system with a synthetic cytokine releaser during pregnancy resulted in abnormal behavioral and pharmacological responses in adult offspring, alterations that parallel characteristics of schizophrenia in humans (Zuckerman, Rehavi, Nachman, & Weiner, 2003; Zuckerman, Weiner, & Weiner, 2005). These data indicate that
inflammation during pregnancy can affect fetal brain development, suggesting that the magnitude of a woman’s inflammatory responses upon exposure to infectious agents may have serious implications for offspring health into adulthood.

**Stress/Depression and Inflammation**

Stress and depression can affect inflammation by at least three physiological pathways. First, chronic stress and depressive symptoms can directly stimulate the production of proinflammatory cytokines, including IL-6 (Glaser, Robles, Sheridan, Malarkey, & Kiecolt-Glaser, 2003; Kiecolt-Glaser et al., 2003). Second, the experience of stress and depression may “prime” an individual to respond in an exaggerated manner upon exposure to psychological stress or biological challenge (Johnson et al., 2002; Maes, Ombelet, De Jongh, Kenis, & Bosmans, 2001). Finally, stress and depression may indirectly increase inflammation by increasing susceptibility to and duration of infectious illness as well as slowing wound healing (Culhane et al., 2001; Glaser et al., 2000; Kiecolt-Glaser, Marucha, Malarkey, Mercado, & Glaser, 1995; Vedhara et al., 1999).

Evidence for the effects of stress and depression on inflammation comes from studies of animals as well as humans. Acute stress in the form of exposure to a novel environment, foot shock, or tail shock induced increases in plasma IL-6 levels in rats (LeMay, Vander, & Kluger, 1990; Zhou, Kusnecov, Shurin, DePaoli, & Rabin, 1993). Further, this physiological response to stress can be conditioned; after repeated shocking, exposure to stimuli that were present when shocks were administered elicited increases in IL-6 (Johnson et al., 2002; Zhou et al., 1993). In addition, rats that had been repeatedly
stressed mounted exaggerated inflammatory responses upon exposure to an antigen, in this case a bacterial endotoxin, compared to rats that had not been exposed to repeated stressors (Johnson et al., 2002). These data indicate that repeated exposure to psychological stress can prime the immune system to respond in an exaggerated manner to psychological and biological challenges.

Mirroring these findings, human studies have found stress and depressive symptoms to be associated with higher circulating levels of inflammatory markers (J. K. Kiecolt-Glaser et al., 2003), as well as exaggerated inflammatory responses to antigen exposure both in vivo and in vitro (Bock, Marsh, & Widdows, 1987). For example, IL-6 increases with age in older populations (Kiecolt-Glaser et al., 2003). However, this increase may be more rapid in those experiencing chronic stress; in a sample of 119 older adults, individuals who were caregiving for a spouse with dementia experienced 4-fold greater increases in IL-6 over a period of 6 years compared to well-matched controls (Kiecolt-Glaser et al., 2003).

In terms of depression, higher levels of circulating IL-6 have been reported in individuals reporting more depressive symptoms as compared to those reporting fewer depressive symptoms (Maes et al., 1995; Penninx et al., 2003; Zorrilla et al., 2001). In addition, at least three studies have found that lymphocytes from depressed individuals show greater inflammatory responses upon in vitro exposure to mitogens (Anisman, Ravindran, Griffiths, & Merali, 1999; Maes, 1995, 1999). Evidence does not allow for a firm statement about the causal direction of the link between inflammation and depression (O'Brien, Scott, & Dinan, 2004). However, substantial evidence indicates that
depression directly affects cytokine production (Kiecolt-Glaser & Glaser, 2002). In addition, cytokine therapy can cause symptoms of depression in humans (Papanicolaou et al., 1998; Yirmiya et al., 2000) and the direct administration of proinflammatory cytokines can induce sickness behavior in animals (Maes, 1995). Therefore, the link between depression and inflammation is likely bidirectional.

Providing additional evidence for the association between depression and inflammation, in a sample of older adults, those who reported more depressive symptoms had higher levels of circulating IL-6 than those who reported fewer symptoms (Glaser et al., 2003). Furthermore, those who reported more depressive symptoms exhibited increases in IL-6 following influenza vaccination; no increase in IL-6 was seen in those reporting fewer depressive symptoms. Symptoms in this sample were, on average, well below a cut-off for clinical depression, indicating that even mild depressive symptoms can predict a primed inflammatory response.

The fact that individuals reporting fewer depressive symptoms in the study by Glaser et al. (2003) experienced no increases in IL-6 following vaccination is consistent with previous data demonstrating that which influenza vaccination does not result in increased IL-6 one month after vaccination (Bernstein, Gardner, Abrutyn, Gross, & Murasko, 1998; Krakauer & Russo, 2001). Therefore, IL-6 increases resulting from influenza vaccination are proposed to indicate dysregulation of normal immune function (Glaser et al., 2003).

Despite the association between stress/depression and inflammation found in animal and human studies, limited research has examined this link during pregnancy.
However, recent data from 30 pregnant women demonstrated that those who reported greater stress exhibited higher levels of IL-6, TNF-α, and lower levels of the powerful antiinflammatory cytokine IL-10 than did women who reported less stress (Coussons-Read et al., 2005). Extending upon these findings, a subsequent study by the same primary investigator demonstrated that maternal stress also predicts exaggerated production of IL-1β and IL-6 by lymphocytes stimulated in-vitro (Coussons-Read, Okun, & Nettles., 2007). In another study of 66 women, those with a history of major depression had higher levels of circulating IL-6 following delivery than did women without this history, suggesting that depression is associated with an exaggerated inflammatory response to this physical and psychological stressor (Maes et al., 2001). These data provide a foundation for further examination of the stress-inflammation link during pregnancy.

**Stress-Buffering Effects of Social Relationships**

Greater social support, measured quantitatively and qualitatively, predicts better psychological well-being, better physical health, and better immune function (Graham, Christian, & Kiecolt-Glaser, 2006a, 2006b). For example, in a sample of 276 healthy individuals, those who reported greater social integration had decreased likelihood of contracting a cold when exposed to a virus (S. Cohen, Doyle, Skoner, Rabin, & Gwaltney, 1997). Similarly, higher perceived support has predicted more robust antibody responses to both Hepatitis B vaccine (Glaser et al., 1992) and influenza vaccine (Pressman et al., 2005) in student populations.
In accordance with the stress-buffering model of social support, relationships may be especially beneficial to mental and physical health when people experience stress (Cohen, 1988). For example, among spouses of those with cancer, individuals who reported higher perceived support had stronger immune function including greater natural killer (NK) cell activity (Baron, Cutrona, Hicklin, Russell, & Lubaroff, 1990); NK cells are a component of the innate immune system (Lanier, 1998). Among medical students, those reporting high levels of social support did not show decreases in NK cell activity seen in their less supported peers in response to the stress of major examinations (Kang, Coe, Karaszweski, & McCarthy, 1998). Among caregivers reporting low support, those who also reported high perceived stress exhibited more negative changes in immune function over a one-year period than did those who reported lower perceived stress (Kiecolt-Glaser, Dura, Speicher, Trask, & Glaser, 1991).

The beneficial effects of social support appear to be independent of personality characteristics, such as extraversion, which may affect the degree to which people seek social relationships (Cohen, 2004). For one, experimental manipulation of support provides benefits. For example, monkeys exposed to the stress of rehousing evinced less detriment in immune function if accompanied by a preferred companion (Coe, 1993).

Although social ties are associated with better health overall, problematic social ties and distressing relationship transitions can be a primary source of stress (Rook, Sorkin, & Zettel, 2004). Demonstrating the negative impact of social loss, immune dysregulation may accompany divorce and the death of a spouse (Bartrop, Luckhurst, Lazarus, Kiloh, & Penny, 1977; Kiecolt-Glaser et al., 1987; Schleifer, Keller, Camerino,
Further, wound healing, an outcome with clear clinical relevance, can be affected by both marital strain and the stress of caregiving (Kiecolt-Glaser et al., 2005; Kiecolt-Glaser, Marucha, Malarkey, Mercado, & Glaser, 1995).

**Social Support and Pregnancy Outcomes.** Although often considered a happy time, the experience of pregnancy itself can be a stressor (Dunkel-Schetter, Gurung, Lobel, & Wadhwa, 2001; Lobel, 1998). Pregnancy is characterized by substantial physiological, emotional, and social changes. Additionally, women may have concerns about their parenting skills, financial demands, or changing work and social roles (Yali & Lobel, 2002). Because pregnancy can be a time of stress or challenge, the potential beneficial and harmful effects of social relationships may be especially evident during this period.

Demonstrating the importance of social support during pregnancy, multiple correlational studies have reported that greater perceived support is associated with fewer symptoms of depression, anxiety, and stress during pregnancy (Berthiaume, David, Saucier, & Borgeat, 1998; Zuckerman, Amaro, Bauchner, & Cabral, 1989). Support from the baby’s father has been the focus of much research; individuals reporting low support from the baby’s father report greater distress and exhibit less prenatal healthcare utilization than their more supported peers (Hobfoll & Liberman, 1987; Kalil, Gruber, Conley, & Sytniac, 1993; MacDonald, Peacock, & Anderson, 1992).

In general, measures of support from the baby’s father are more predictive of perinatal outcomes than are measures of support from the family. However, family support may be especially important for certain women. In particular, studies of pregnant
teenagers have found that women who reported greater family support had higher birth weight babies, experienced fewer neonatal complications, and reported less postpartum depression (Boyce, Schaefer, & Uitti, 1985; Turner, Grindstaff, & Phillips, 1990). In addition, women of certain ethnicities, including African-American women and Latinas, may rely on extended family for support to a greater extent than do European-American women (Sagrestano, Feldman, Killingsworth Rini, Woo, & Dunkel-Schetter, 1999).

As noted, a discussion of social relationships would not be complete without an acknowledgement of negative social interactions. Data regarding support networks of pregnant women indicates that sources of support are frequently also sources of interpersonal tension, frustration, criticism, and conflict (Cramer & McDonald, 1996; Rhodes & Woods, 1995). Particularly disturbing is the rate of physical and sexual violence towards pregnant women which is estimated to affect 9-20% of all pregnancies (Gazmararian et al., 1996). Physical and emotional abuse are believed to be more commonly experienced by pregnant women than women in general, particularly women with unwanted pregnancy (Gazmararian et al., 1995). Violence during pregnancy has been associated with risk for low birth weight, infections, and greater likelihood of smoking and alcohol use during pregnancy (McFarlane, Parker, & Soeken, 1996). Moreover, a study of 401 pregnant women, risk of preterm labor was 4.1 times greater among women reporting the experience of severe violence as compared to women reporting no abuse (Shumway et al., 1999). In sum, close relationships can have substantial impact, both positive and negative, on maternal mental health, maternal health behaviors, and birth-related outcomes.
Current Study

Substantial evidence links a) maternal stress/depression to negative perinatal outcomes, and b) inflammatory immune responses to negative perinatal outcomes. Furthermore, strong data from non-pregnant populations demonstrates that stress and depression are associated with inflammation. Despite these well-established links, limited research has attempted to identify inflammation as a mediator of the association between stress/depression and perinatal outcomes. In addition, the potential buffering effects of social support within this model are unknown.

The current study will prospectively examine the relationship between psychosocial factors and inflammation during pregnancy both at baseline and in response to antigen exposure. Pregnant women will complete questionnaires and have blood drawn at two timepoints: baseline and one week after receiving influenza vaccination. Key variables of interest are perceived stress, depressive symptoms, social support, and levels of the proinflammatory cytokine IL-6. This study will be the first to examine inflammatory responses to \textit{in vivo} antigen challenge during pregnancy.

It is hypothesized that both stress and depressive symptoms will predict inflammation. Specifically, it is predicted that women reporting greater stress or depressive symptoms will have higher circulating levels of IL-6 at baseline. It is also predicted that women reporting greater stress or depressive symptoms will experience significant increases in IL-6 one week following vaccination, while women reporting fewer stress or depressive symptoms will evidence no increase in IL-6 in response to vaccination. Further, it is expected that the relationship between stress and inflammation
will be partially mediated by depression; that is, it is believed that stress will exert some of its effects by increasing depressive symptoms. Finally, it is hypothesized that social support will buffer the effects of stress on depressive symptoms and inflammation.

Among women reporting high stress, those who also report low levels of spousal and familial support are predicted to have more depressive symptoms and higher levels of IL-6 at both timepoints than their more supported counterparts.

In sum, findings from this study will address two pathways by which stress or depressive symptoms can affect inflammation. By examining levels of circulating IL-6 prior to vaccination, this study will contribute to emerging data demonstrating direct effects of stress and depression on inflammation during pregnancy. These data alone are meaningful. However, the truly innovative aspect of the current investigation is the assessment of IL-6 after vaccination. This study will be the first to determine whether women reporting stress or depressive symptoms during pregnancy respond to biological threats, such as viruses, in a manner that is exaggerated and potentially dangerous to the developing fetus and mother.
CHAPTER 2
STUDY DESIGN AND METHODS

The purpose of the current investigation was to examine relationships among psychosocial factors and inflammation during pregnancy both at baseline and in response to influenza vaccination. Data from the current study were collected as part of a larger investigation. In particular, Albert Franco, MD, fellow of Obstetrics and Gynecology examined IL-6 in cervicovaginal fluid samples. This larger data set, outlined in Table I, will provide opportunity for examining additional outcomes in the future.

<table>
<thead>
<tr>
<th>Timeline</th>
<th>Data Collection</th>
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<tr>
<td>Baseline (vaccination)</td>
<td>• Demographic information</td>
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<tr>
<td></td>
<td>• Clinical information and psychosocial questionnaires</td>
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<td>• Cervicovaginal fluid sample</td>
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<td></td>
<td>• 50ml blood sample</td>
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<tr>
<td>1-2 weeks post-vaccine</td>
<td>• Clinical information and psychosocial questionnaires</td>
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<td></td>
<td>• Cervicovaginal fluid sample</td>
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<td>• 50ml blood sample</td>
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<td>Post-delivery chart review</td>
<td>• Gestational age at delivery</td>
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<td>• Birth weight</td>
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<td>• Blood pressure at regular perinatal visits</td>
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As depicted in Table II below, data used in the current study was from 2 timepoints: baseline and 1-2 weeks after influenza vaccination. Of note, analyses of IL-6 in the current investigation required only a portion of the total blood samples taken at
baseline and follow-up. Therefore, it will be possible to examine additional outcomes in the future, including cytokines that are not included in the current investigation.

**Table II: Current Study Data Collection Timeline**

<table>
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<th>Timeline</th>
<th>Data Collection</th>
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<tr>
<td>Baseline (vaccination)</td>
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<td>• Clinical information and psychosocial questionnaires</td>
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<td>• 10ml blood sample</td>
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<td>1-2 weeks Post-vaccination</td>
<td>• Clinical information</td>
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<td>• 10ml blood sample</td>
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**Participants and Recruitment**

Sixty pregnant women ages 18 and older were recruited. The majority of women were recruited from the Ohio State University (OSU) General Perinatal Medical Clinic which provides services to a large number of women of lower socioeconomic status. No upper age limit was selected to allow subject recruitment to be as inclusive as possible. However, the final subject age range was 18 to 37 years of age (mean age = 25 years, SD=4.8). The majority of women were in the first (n=31) or second (n=25) trimester of pregnancy at baseline (0-28 weeks).

Women were recruited from October through mid-April 2005-2007 to coincide with the recommendations for influenza vaccination for pregnant women described below. Women with fetal anomaly were excluded from participation. Women were excluded from participating in the vaccination portion of the study if they had already received influenza vaccination during the current influenza season. Two women who had already received the influenza vaccination participated in the baseline session only. Both had received their vaccination more 12 weeks prior to completing their baseline session.
Women were excluded from participation if they reported that their fetus had known health complications. Two women admitted to the study had twin pregnancies. Data from these two participants were excluded from analyses related to gestational age at delivery and blood pressure since multifetal gestation affects these outcomes. One woman who had chronic hypertension was admitted to the study. Her data were excluded from analyses related to blood pressure.

**Influenza Vaccine**

Pregnant women are more likely than the general population to experience serious consequences if they contract influenza. Fortunately, influenza vaccination is safe during pregnancy. For these reasons, both the American College of Obstetrics and Gynecology (ACOG) and the Centers for Disease Control and Prevention (CDCP) recommend that all women who will become pregnant during influenza season (October through mid-May) be vaccinated (American College of Obstetricians and Gynecologists, 2004; Harper, Fukuda, Uyeki, & al., 2004). Because influenza vaccination is safe and recommended, it provides an excellent model for studying immune responses to antigen exposure during pregnancy.

**Questionnaire Data**

Described in detail below, questionnaire measures were used to assess demographic and psychosocial factors of interest at baseline. Moreover, clinical information (e.g. blood pressure, weight, medications) were collected via chart review at baseline and 1-2 week follow-up.
Screening Criteria. To determine eligibility for the study, each woman was verbally asked the following screening questions by a researcher: 1) Have you received an influenza vaccination (flu shot) during the current flu season (dates were provided by the researcher)? 2) Are you having twins or triplets? 3) Do you have any reason to believe that your baby has a health problem? 4) Have you ever been diagnosed with high blood pressure? Information regarding each of these variables was confirmed via chart review and recorded in the Baseline Staff Packet, described below.

Demographic and Background Information. Each participant was asked to provide information regarding her age, ethnicity, education level, marital status (single, divorced/separated, married, in a long-term relationship), income, and employment status. Women were also asked to estimate their height and pre-pregnancy weight.

Health Behaviors. Because health behaviors may differ based on stress and depression, information regarding alcohol use, exercise, and sleep were collected. Specifically, women were asked about their alcohol consumption in the past 48 hours, the frequency and duration of physical activity in the past week, and the number of hours of sleep in the previous night and previous 3 nights. Moreover, women were asked how many days per week they take a prenatal vitamin. As a confirmation that women were eligible for the study, they were asked whether they have received an influenza vaccination this season. Because exposure to previous vaccination may affect antibody responses to subsequent vaccination, women were also asked if they received influenza vaccination in the previous flu season. This information is needed for possible future analyses of antibody titers.
Subjective Socioeconomic Status (SES). In addition to objective measures of SES (e.g., education, income), a measure of subjective SES was included. Participants were presented with a drawing of a ladder with 10 rungs. Each rung represents “where people stand in our society” with those at the top of the ladder possessing the most money, education, and the best jobs (Adler, Epel, Castellazzo, & Ickovics, 2000). Participants were asked to indicate where they believe they fall relative to others in society by selecting a rung on the ladder. The relationship between subjective SES and objective markers of SES may differ across ethnicities. In a diverse sample of 878 pregnant women, objective SES was less predictive of subjective SES among African-American women than among Caucasian-American women (Ostrove, Adler, Kupperman, & Washington, 2000). Moreover, subjective SES was a significant predictor of self-rated health among women of Caucasian-American and Asian-American ethnicity after controlling for objective SES indicators (Ostrove et al., 2000).

The Center for Epidemiological Studies Depression Scale (CES-D). This measure has been used extensively as a brief measure of depressive symptomatology (Basco, Krebaum, & Rush, 1997; Radloff, 1977). Studies indicate acceptable test-retest reliability and excellent construct validity (Basco et al., 1997). Although the CES-D is a valid and reliable measure of depressive symptomatology, it does not allow for diagnosis of clinical depression. It is estimated that 40-50% of those who score at or above a cut-off of 16 points on the CES-D meet criteria for major depressive disorder as determined by clinical interview (Weissmann, Sholomskas D., Pottenger M., Prusoff, & Locke, 1977). Of note, even subclinical depressive symptoms predict altered immune function (Glaser et al.,
2003) as well as increased risk of mortality (Steptoe, Wardle, & Marmot, 2005). In relation to pregnancy, elevated depressive symptoms, as indicated by CES-D scores, are associated with negative outcomes including restricted fetal growth (Hoffman & Hatch, 2000), spontaneous preterm birth (Orr, James, & Blackmore Prince, 2002), and impaired neuromotor performance among neonates (Lundy et al., 1999).

**The Perceived Stress Scale (PSS).** The 4-item version of this scale was used to measure the subjective experiences of stress and coping with stress using the past month as timeframe. This scale, which shows adequate internal reliability and predictive validity, is a useful brief measure of stress (Cohen, Kamarck, & Mermelstein, 1983c). Furthermore, the PSS measures a construct that is independent of depressive symptomatology (Cohen et al., 1983c). Demonstrating predictive validity in pregnant populations, in a sample of 454 women, those who reported greater scores on the 14-item version of the PSS had greater likelihood of contracting bacterial vaginosis than did those who reported lower scores (Culhane et al., 2001).

**The Pregnancy-Specific Anxiety Questionnaire (PSAQ).** This measure, developed to assess anxiety about pregnancy (Roesch et al., 2004), asks women “How have you felt about being pregnant in the past week, including today?” with regards to feeling anxious, concerned, afraid, and panicky. Ratings are on a scale from 1 (not at all) to 5 (very much). This measure has adequate internal reliability and was predictive of corticotrophin-releasing hormone levels and gestational age at delivery in a sample of 282 pregnant women (Mancuso, Dunkel-Schetter, Rini, Roesch, & Hobel, 2004).
Parental Attitudes. Two questions adapted from the National Survey for Family Growth were used in the current study (Brown & Eisenbert, 1995). Each participant was asked to describe how she felt about her pregnancy at the moment of discovery from 1 to 10. Scores between 1-3 indicate unhappiness, 4-7 indicate ambivalence, and 8-10 indicate happiness. Each participant also rated her perception of the father’s happiness using the same scale. Previous research using these two items found that parental feelings of happiness about pregnancy were associated with birth weight; women who perceived their partners to be happier about the pregnancy than themselves were more likely than other women to give birth to babies of low birth weight (Keeley et al., 2004).

Interpersonal Support Evaluation List (ISEL). General social support was measured using a short version of the ISEL. This 12-item measure assesses three types of social support: appraisal, tangible, and belonging (Cohen & Hoberman, 1983b). The ISEL is widely used and demonstrates excellent test-retest and internal reliability. In addition, ISEL scores predict less depressive symptomatology (Cohen, Mermelstein, Kamarck, & Hoberman, 1985). As reviewed, perceived support during pregnancy predicts various mental health and behavioral outcomes including depressive symptoms, health care utilization, and birth weight (Collins, Dunkel-Schetter, Lobel, & Scrimshaw, 2004).

Baby’s Father and Family Support. Additional items were used to assess social support provided specifically by the baby’s father or partner (8 items) and the family (parents, siblings, children, and in-laws; 7 items). These questions were adapted by Feldman et al. (2000), from a study with pregnant teenagers (Turner et al., 1990). Both
scales show good reliability (Feldman, Dunkel-Schetter, Sandman, & Wadhwa, 2000). Turner et al. (1990) found family support to be predictive of birth weight in a sample of 268 pregnant teenagers. Similarly, Feldman et al. (2000) demonstrated that a model using these two measures and the 40-item version of the ISEL predicted birth weight in a sample of 247 women ages 18-40.

**Test of Negative Social Exchange (TENSE).** To provide a measure of negative or upsetting social experiences, a modified version of the TENSE was used (Ruehlman & Karoly, 1991). Specifically, 2 subscales from the TENSE were administered, those measuring hostility/impatience and insensitivity. The TENSE subscales have good test-retest reliability, internal consistency, and convergent and discriminant validity (Ruehlman et al., 1991). Higher scores on the TENSE predicted greater reporting of health symptoms (Edwards, Hershberger, Russell, & Markert, 2001) as well as greater negative mood (Finch, 1998) in college-aged samples. In a daily diary study of 45 men and 55 women (average age of 34 years), higher scores on the TENSE predicted greater negative mood (Mohr et al., 2003). Moreover, women in this study experienced more lasting negative mood as a result of negative social interactions than did men (Mohr et al., 2003), suggesting that assessment of social conflict may be especially useful in female populations.

**Staff Packet.** The following information was collected via chart review at baseline to confirm that the participant met exclusionary criteria and to provide data for analysis purposes: smoking status, history of diabetes, history of hypertension, fetal anomaly, multifetal gestation. At baseline only, the number of previous pregnancies (gravidity),
number of previous births (parity), and history of preterm birth and preeclampsia was recorded. Finally, the following information was recorded at each visit, using chart review where applicable: date of visit, time of visit, time of blood draw, expected delivery date, height and weight, blood pressure, current mental and physical health conditions, and medication usage.

**Blood Data**

Whole blood was collected into five 10mL vacutainers while the subject was in a seated position. To control for diurnal variation, all samples were collected between 8:30 – 1:30 pm. Samples were immediately centrifuged and placed in -80 C degree freezer storage until analysis. Serum levels of IL-6 were assayed in duplicate using ultra sensitive multiplex kits from Meso Scale Discovery (MSD). MSD ultra sensitive multiplex kits demonstrate good intra-assay precision and inter-assay precision (MSD Catalog; Product Number K11025C-2). Although kits have good precision, to control for potential variation between assay kits, samples were batched by subject.

**Perinatal Outcome Information**

In addition to data collected for the current investigation, perinatal outcome information collected as part of the larger study was examined in exploratory analyses. Specifically, information regarding three perinatal health outcomes was collected via chart review: blood pressure, length of gestation, and birth weight. Blood pressure from each regular perinatal visit was recorded by nursing staff in patient medical records as
part of regular perinatal care. For the purposes of the current study, the highest systolic and diastolic blood pressure recorded was used for analytic purposes. Length of gestation and birth weight were also collected.

The collection of information related to these outcomes, while very useful, was not necessary to accomplish the goals of the current investigation. Instead, information related to these variables was intended to provide preliminary data for the development of future studies. Therefore, no efforts were made to collect data from women after the 1-2 week follow-up that occurred for the full study. Moreover, for the purposes of the proposed exploratory analyses linking psychosocial factors to these outcomes, only baseline questionnaire data were utilized.

Analyses of preterm birth and low birth weight were conducted using categorizations based on clinical cut-offs. Preterm birth was defined as gestational age of less than 37 weeks and low birth weight as equal to or less than 5 pounds 8 ounces (2,500 grams; (Kramer, 1987). Because only 1 participant met criteria for gestational hypertension during her pregnancy (i.e., 140/90, Page, 2002), blood pressure data were analyzed continuously rather than categorically.

Procedure

Women were recruited primarily face-to-face at The Ohio State University General Perinatal Clinic located on the 2nd floor of Cramblett Hall. At this clinic, women who have requested appointments for their first prenatal visit were scheduled for Monday morning appointments (8:30 AM – 12:00 PM). While waiting for their appointments,
women were approached by Albert Franco, MD (fellow of Obstetrics and Gynecology) or myself. We verbally described the study and asked the woman if she was interested in participating. If she was interested in participating, the woman was asked screening questions. If she was eligible based on her responses, she was provided a copy of the written informed consent for her review. After giving her time to review the informed consent, we returned to answer any questions the woman had about participation. If she was still interested in participating, she was asked to sign the informed consent and provided with a copy of the consent for her own records.

Next, the participant was provided with questionnaires to complete. Questionnaires required approximately 20-25 minutes to complete. The majority of participants had adequate time to complete questionnaires while waiting for their appointments or waiting for blood draws for their regular care. When a participant was seen by her physician for her appointment, it was determined if a blood draw was needed for her care. The majority of participants required a blood draw because this was the first prenatal appointment. If a blood draw was needed, the woman was directed to the phlebotomy laboratory in the 1st floor of Cramblett Hall. At that time, she was provided with 5 labeled vacutainers for collection of blood for the study. The participant took these collection tubes with her to the phlebotomy laboratory so that blood sample for the study could be collected at the time that her usual care samples were drawn. In this way, the woman was required to have a needle stick only once. The woman then returned to the 2nd floor clinic with these blood samples where they were given to the researchers. If the
participant did not require a blood draw for her usual care, blood was drawn by Dr. Albert Franco or by Clinical Research Center nursing staff in the Cramblett Clinic.

After completing the blood draw, the participant was given an influenza vaccination by a nurse in the Perinatal Clinic. She was then scheduled for her follow-up study visits. At the follow-up visits, blood and questionnaire data were collected using the same procedure. Moreover, the date that the woman was due for delivery was recorded. The researchers conducted follow-up via chart review to determine the actual date of delivery, birth weight, and blood pressure at regular prenatal visits.

When possible, visits for the study were scheduled to coincide with regular care visits to reduce subject burden. When a woman was seen for the study at the time of a regularly scheduled visit, the total time of her participation was difficult to estimate because data collection coincided with regular care. When a woman was seen solely for study purposes, her participation time at each visit was approximately 35-50 minutes. Participants received $15.00 in cash as compensation for their time at each visit as well as a parking pass.

**Power Analysis**

In a study of older individuals (mean age 71 years), an interaction between depressive symptoms and time was found (pre-vaccine versus post-vaccine; $F(1,116) = 7.42, p = .007$; (Glaser et al., 2003). Analysis of preliminary data from the current study indicated that the level of depressive symptoms seen in the current sample was higher than that reported in the sample from Glaser et al. (2003). Specifically, among the 60
women who completed the CES-D at baseline, 31 women (52%) scored above at or above the clinical cut-off of 16 for depressive symptomatology. In comparison, in the sample from Glaser et al., 29 of 119 (24%) of the sample scored at or above a clinical cut-off of 5 on the Beck Depression Inventory – Short Form (BDI-SF).

Based on this preliminary data, it was expected that the greater incidence of depression in the current sample would increase the ability to detect effects of depression. To determine power needed to see effects of higher depression scores, analyses of the dataset from the study by Glaser and colleagues were done to compare individuals scoring 7 or above on the BDI – SF (n = 14) to individuals scoring below this cut-off (n = 105).

ANCOVA analyses were conducted to examine the difference between IL-6 levels post-vaccination in those above versus below this cut-off after controlling for baseline levels of IL-6. ANCOVA analyses revealed a significant difference between groups at follow-up ($F(1,116) = 11.02, p = 0.001$). Post vaccination levels of IL-6 were .74 (SD = .32) log(pg/ml) among those who scored at or above this cut-off. In comparison, post-vaccination levels of IL-6 were .57 (SD = .24) log(pg/ml) among those who were below this cut-off.

Using these ANCOVA results, the following formula was utilized to calculate the effect size: $\beta / [\sigma * \sqrt{1-r^2}]$. An effect size of .91 in terms of Cohen’s $d$ was determined. Next, the following formula was used to calculate the needed sample size: $[2(1-r^2)(1.96+.842)^2] / ([\beta/ \sigma]^2]$. Results indicated that with .80 power and .05 $\alpha$, 38 individuals would be needed to see this effect. [Note: In this formula, the value of 1.96
reflects the α level of .05. Specifically, 1.96 is the 97.5\textsuperscript{th} percentile of the standard normal distribution. The 97.5 percentile was used instead of the 95\textsuperscript{th} because this is a two-sided test. The value of .842 is the 80\textsuperscript{th} percentile of the standard normal distribution and reflects a power level of 80%.

Additional analyses were conducted based on research with a sample of 30 pregnant women. This study reported statistically significant correlations between self-reported stress and circulating proinflammatory cytokines IL-6 (\(r = 0.349, p < .01\)) and TNF-\(\alpha\) (\(r = 0.704, p < .01\) (Coussons-Read et al., 2005). The same study reported an inverse relationship between self-reported stress and the anti-inflammatory cytokine IL-10 (\(r = -0.519, p < .01\); (Coussons-Read et al., 2005). To detect the most conservative of these effects (\(r = 0.349\)) with .80 power and .05 α, a sample of 46 would be needed for a one-tailed analysis while a sample of 59 would be needed with a two-tailed analysis. Therefore, to provide the most conservative power estimation for the current study, the targeted sample size was 59 women at baseline and 38 at follow-up. This was expected to provide sufficient power to detect effects of stress/depression on IL-6 at baseline, as well as power to detect differences in IL-6 responses to influenza vaccination based on depressive symptoms.

**Data Analysis**

**Descriptive Statistics.** Height and self-reported pre-pregnancy weight were used to calculate pre-pregnancy body mass index (BMI), calculated as weight divided by height squared (BMI = kg/m\(^2\)). Next, descriptive statistics including means and standard
deviations or frequencies were calculated for the following demographic measures: pre-pregnancy BMI, age, gestational age, race, marital status, parity, gravidity, education level, income, and employment status. Descriptive statistics (means and standard deviations) were also calculated for each psychosocial measure: CES-D, PSS, PSAQ, Parental Attitudes (mother and father), ISEL, Baby’s Father Support, Family Support, TENSE subscales (hostility and insensitivity), and subjective socioeconomic status. Finally, descriptive statistics were calculated for health behavior variables: alcohol consumption, participation in regular exercise, hours of sleep in the past night, hour of sleep in then past 3 nights, and prenatal vitamin usage (days per week).

**Associations among demographic variables.** Pearson’s product-moment correlations were used to examine the relationship between the following demographic variables: BMI, age, education, income, gravidity, and parity. It was predicted that age would be positively associated with BMI, education, income, gravidity, and parity.

**T-tests were used to determine group differences in demographic characteristics (BMI, age, gravidity, and parity) based on marital status.** It was predicted that women who are married would be older and greater gravidity and parity than unmarried women. Finally chi-square analyses were used to examine whether women who were married were more likely to report greater income and education.

**Associations among demographics, psychosocial variables, and health behaviors.** Although psychosocial questionnaire data were collected at both baseline and follow-up, for the current investigation, only the baseline measures were used in analyses. This analytic strategy was utilized because the goal was to examine potential priming of the
inflammatory immune response. Therefore, information about mood and social support in the period preceding vaccination is most relevant.

First, Pearson’s product-moment correlations were conducted to examine the relationships between demographic and psychosocial variables. It was expected that income, education would be negatively associated with scores on the CES-D, PSS, and PSAQ and subjective SES. Further, it was hypothesized that women who reported less social support (measured by the ISEL, Baby’s Father Support Scale, and Family Support Scale) would have higher scores on the CES-D, PSS, and PSAQ. Similarly, correlations tested whether those reporting greater social conflict (as measured by the TENSE), and greater ambivalence about being pregnant (as measured by the Parental Attitudes Scale), had higher scores on the CES-D, PSS, and PSAQ.

Next, analyses were conducted to examine relationships between demographic variables (BMI, age, education, and income) and health behaviors (exercise, prenatal vitamin use, sleep, and smoking). Correlational analyses were utilized for sleep, while t-test analyses were used for exercise (regular versus not), prenatal vitamin use (regular versus not) and smoking status (current smoker versus not). No woman reported consuming alcohol within 48 hours of any visit.

Analyses were also conducted to determine if women reporting greater stress or depressive demonstrated differences in health behaviors. Specifically, product-moment correlations were conducted between scores on the CES-D and PSS on the one hand and sleep on the other. In addition, t-tests were conducted to examine potential difference in
stress and depression based on exercise (regular versus not), prenatal vitamin use (regular versus not) and smoking status (current smoker versus not).

Finally, correlational analyses were conducted to examine relationships among psychosocial variables. Analyses were also conducted to examine moderation and mediation effects. For example, analyses examined whether social support moderated the effect of perceived stress on depressive symptoms.

**Associations between demographics, health behaviors, and baseline IL-6.**

Correlational analyses and t-tests were used to examine associations between demographic characteristics and health behaviors on one hand and baseline IL-6 on the other. These analyses were used to identify possible confounding variables that could affect subsequent analyses of psychosocial correlates of inflammation.

**Testing of primary hypotheses.** It was hypothesized that levels of IL-6 pre- and post-vaccination would be predicted by stress and depressive symptoms. Further, it was expected that the relationship between stress and inflammation would be partially mediated by depressive symptoms. That is, it was believed that a primary pathway by which stress affects inflammation is by increasing depressive symptoms that, in turn, increase inflammation.

To test hypotheses related to relationships between psychosocial factors and baseline inflammatory markers, regression analyses were utilized. Baseline levels of IL-6 were used as the dependent variable and each relevant psychosocial factor was tested as an independent variable in separate models.
Next, to test hypotheses related to inflammatory responses from pre- to post-vaccination, a repeated measures regression model was used (repeated-measures generalized linear model; SPSS 13.0 for Windows, SPSS Inc, Chicago, Ill). This procedure generalizes the standard multiple regression model to incorporate repeated measures of dependent variables (Cohen & Cohen, 1983a; Keppel & Zedeck, 1989). The IL-6 data were subjected to a logarithmic transformation with a base of 10 to normalize the distributions before analysis.

First, the predictive value of depressive symptoms and perceived stress were tested individually. In separate models, CES-D and PSS scores were used as continuous independent variables with IL-6 (repeated across time) as the dependent measure. Additional mediational analyses were planned if significant effects for both perceived stress and depression were found. Specifically, the repeated-measures general linear model would be used. PSS scores would be entered as the between subjects variable and CES-D scores as a covariate. If depression was a mediator of the relationship between PSS and inflammation, the predictive value of stress for inflammation should be reduced significantly.

Furthermore, to test whether social support buffered the effects of stress, tests of the moderating effects of social support were planned. Specifically, the following interaction terms were created: PSS X ISEL, PSS X Baby’s Father Support, and PSS X Family Support. Analysis were then conducted using each individual independent variable (PSS and support measures) and these interaction terms in the same model to predict depressive symptoms and measures of inflammation.
Exploratory Analyses. As described, this study was designed and powered to examine inflammatory markers at baseline and in response to influenza vaccination. Analyses were also conducted to examine links between psychosocial variables, inflammation, and perinatal outcomes (i.e., birth weight, gestational hypertension, and length of gestation). These analyses were exploratory and were not the primary focus of the current study; base rates of these outcomes are not large enough to provide adequate statistical power in the current investigation. For example, gestational hypertension affects 5-10% of U.S. pregnancies (Granger et al., 2002).

The goal of these exploratory analyses was to examine whether the data suggest that inflammation mediates the relationship between stress and the three perinatal outcomes assessed: birth weight, gestational length, and maternal blood pressure.

To examine mediation, a three-step procedure was used as described by Baron & Kenny (1986). First, analyses were conducted to determine if stress and inflammation predicted each perinatal outcomes. Specifically, for the categorical variables of preterm delivery and low birth weight, t-tests were conducted to compare groups on each psychosocial variable (e.g., perceived stress, depressive symptoms, and pregnancy-specific anxiety). For blood pressure, multiple regression analyses were conducted using the same psychosocial variables as predictors. Analyses were repeated using SES indicators (income, education, and subjective SES), parity, and marital status as covariates. In sum, these analyses were used to determine whether negative affect predicted any of the perinatal outcomes and, if so, whether this predictive value remained after controlling for potential confounds.
Next, univariate ANOVA analyses were conducted to compare levels of IL-6 among those with normal versus low birth weight babies and term versus preterm deliveries. Similarly, regression analyses were used to examine the relationship between IL-6 and blood pressure. Analyses were repeated controlling for age and pre-pregnancy BMI. These analyses were used to determine whether inflammation predicted any of the perinatal outcomes.

Finally, if statistically significant relationships were found between a) stress and perinatal outcomes and b) inflammation and perinatal outcomes, further analyses were planned to determine if inflammation mediated the relationship between stress and perinatal outcomes. For categorical variables, binary logistic regression analyses were planned in which the predictive psychosocial measure(s) as well as IL-6 would be entered as independent variables. For blood pressure data, multiple stepwise regression analyses were planned in which the predictive psychosocial measure(s) as well as the predictive inflammatory measures would be entered. If the psychosocial variable was no longer a significant predictor when the inflammatory measure is included in the model, this would indicate that inflammation mediated the relationship between the psychosocial variable and the perinatal outcome.
CHAPTER 3

RESULTS

Descriptive Statistics

Demographic Characteristics. A total of 60 women provided data at baseline. Demographic and descriptive data are presented in Table 1. The average age of participants was 25.24 (SD = 4.89) years. Participants ranged in age from 18 to 37 years. Upon enrollment in the study, 31 women were in their first trimester of pregnancy, 24 were in their second trimester, and 5 were in their third trimester. Women completed the baseline visit at an average of 15 weeks (SD = 7.8) weeks of pregnancy. The average pre-pregnancy BMI was 27.78 (SD = 6.08). This self-report is believed to be adequately accurate, as BMI calculated using weight at baseline visit was 28.54 (SD = 6.72) and pre-pregnancy BMI and BMI by scale at the baseline visit were highly correlated (r = .94, p < .001).

The majority of participants (n=34) identified themselves as African/African-American. In addition, 19 identified themselves as White/Caucasian, 1 Asian, and 6 as biracial (3 African-American and Caucasian; 3 Native American and Caucasian). One participant indicated that she was of Hispanic origin.
The majority of participants (n=46) were unmarried. Among the 14 married women, the average length of marriage was 6.3 years (SD = 4.68). In terms of education, 19 women reported completing less than high school, 30 reported completing high school, and 11 reported education past high school.

Twenty-five women reported being currently employed and 35 reported being unemployed. The majority of women (n=38) reported a total family income of less than $15,000 per year. In addition, 14 reported a family income of $15,000-$29,999 per year and 6 reported an income of $30,000 or greater per year. Two women did not respond to this question. Information regarding pregnancy history was collected via chart review. On average, women had an average of 2.13 (SD = 1.77) previous pregnancies and 1.4 (SD = 1.18) previous births.

Psychosocial Variables. Means and standard deviations were calculated for each of the major psychosocial measures utilized (Table 2). Scores on the CES-D ranged from 1-41 with an average score of 17 (SD = 10). Thirty-one women (52%) scored at or above a clinical cut-off of 16 on the CES-D. Scores on the Perceived Stress Scale (PSS) ranged from 0-13 with an average of 6.65 (SD = 2.82).

Scores on the Pregnancy Specific Anxiety Questionnaire ranged from 4 to 18. Women reported an average of 10.37 (SD = 3.96) on this scale. Means (standard deviations) for individual PSAQ items were as follows: Anxious 2.91 (1.49); Concerned 3.33 (1.37); Afraid 2.25 (1.44); Panicky 1.88 (1.17).

Three scales were used to assess social support. Women had an average score of 38.75 (SD = 7.25) on the ISEL. Women scored an average of 3.23 (SD = .91) on the
of negative social interactions, women had an average score of .65 (SD = .73) on the hostility subscale and an average score of .78 (SD = .95) on the insensitivity subscale.

In terms of happiness about pregnancy, women reported an average of 6.93 (SD = 3.13) on a 10-point scale regarding their own happiness when they discovered they were pregnant. They reported an average of 7.87 (SD = 2.59) regarding their beliefs about how happy the father of the baby was when he found out about the pregnancy. The women’s self-reported happiness scores were also divided into tertiles to reflect unhappiness (1-3), ambivalence (4-7) and happiness (8-10) about their pregnancy. When divided in this way, 32 women were classified as happy, 16 as ambivalent, and 12 as unhappy. In terms of partner happiness, 37 women rated their partner as happy, 18 as ambivalent, and 5 as unhappy. Overall, 27 women (45%) rated both themselves and their partner as happy about the pregnancy.

Finally, subjective socioeconomic status ranged from 1-8 on a 10 point scale with higher values indicating greater perceived status. The average score on this measure was 4.7 (SD = 1.7).

**Health Behaviors.** Information regarding health behaviors is presented in Table 3. No participant reported alcohol consumption in the previous 48 hours. The majority of women denied current cigarette smoking (n = 45) while 15 women endorsed current smoking. Twenty-eight women reported that they did not participate in vigorous physical activity at least once per week. Twenty-four women reported that they did such activity. Eight women did not complete this question. Women reported sleeping an average of
6.65 (SD = 1.8) hours in the previous night, and 21.10 (SD = 6.66) hours in the previous
3 nights. Nine women did not complete questions regarding sleep. Finally, 72% (n=43) of
women reported taking prenatal vitamins daily. In addition, 20% (n=12) reported taking
no prenatal vitamins. Three women reported sporadic use (1-5 times per week). Two
women did not answer this question.

Demographic, Psychosocial, and Health Characteristics of Completers Versus
Noncompleters. Of the 60 women who completed the baseline session, 37 completed
follow-up at 1-2 weeks post-vaccination. Of interest was whether those who completed a
follow-up session differed significantly from those who did not in terms of demographic,
psychosocial, or health characteristics. T-tests and chi-square analyses were conducted to
compare the 37 follow-up completers to the 23 women who were noncompleters.

In terms of demographic characteristics, t-tests demonstrated that women who
completed both baseline and follow-up (n=37) did not differ from those who completed
baseline only (n=23) in age (t(58) = .88, p = .34), BMI prior to pregnancy (t(58) = .09, p
= .96), or number of previous pregnancies (t(58) = .12, p = .91). Chi-square analyses
demonstrated that completers did not differ from noncompleters in likelihood of being
married ($\chi^2 (1,60) = .16, p = .69$), race (African-American versus Caucasian) ($\chi^2 (1,53) =
.34, p = .53$), education (less than high school versus high school graduate or greater ) ($\chi^2
(1,60) = .54, p = .46$), or income categorization ($\chi^2 (2,58) = 3.8, p = .15$). Finally, on
average, completers were at a greater gestational age (16 weeks, SD=8.5) at their baseline
session than were noncompleters (12 weeks, SD=4.9), t(58) = -2.0, p = .05.
Analyses comparing completers to noncompleters in terms of psychosocial characteristics revealed no significant differences in scores on the CES-D, PSS, PSAQ, TENSE, ISEL, BFSS, or FSS (ps ≥ .14). The average score on the CES-D was 15.6 (SD=10) among noncompleters and 18.5 (SD=10) among completers. As compared to women who did not complete a follow-up session, those who did complete a follow-up session were significantly less happy about their pregnancies (t(58) = 4.5, p <.01), perceived their partners to be significantly less happy about their pregnancies (t(58) = 3.4, p <.01), and reported significantly lower subjective SES (t(57) = 3.2, p <.01).

Finally, in terms of health behaviors, completers did not differ from noncompleters in smoking status ($\chi^2 (1,60) = .01, p = .96$), sleep in the past 24 hours (t(49) = -.49, p = .63), sleep in the past 3 days (t(49) = -.29, p = .77), regular use of prenatal vitamins ($\chi^2 (1,58) = .14, p = .71$), or likelihood of endorsing regular vigorous physical activity ($\chi^2 (1,52) = 1.82, p = .18$).

In sum, women who completed a baseline session as well as follow-up study visit were very similar to those who completed a baseline session only. Those who completed a follow-up were at a significantly greater gestational age at baseline, less happy about their pregnancies, perceived their partners to be less happy about their pregnancies, and reported lower subjective SES. It is possible that women who were less happy about their pregnancies and had lower subjective SES were more motivated to complete a follow-up visit because of reimbursement for participation as well as the support provided from the research staff.
**Associations Among Demographic Variables**

Pearson’s product-moment correlations were conducted to examine relationships among demographic variables (BMI, age, education, income, gravidity, and parity; Table 4). Age was predictive of greater education ($r = .48, p < .001$), greater income ($r = .48, p < .001$), greater number of previous pregnancies ($r = 0.36, p = 0.004$) and greater number of previous births ($r = 0.27, p = 0.03$), but was not related to self-reported BMI prior to pregnancy ($r = .18, p = 0.18$). As expected, education and income were positively correlated ($r = 0.62, p < 0.001$). Body mass index, education, and income were not associated with the number of previous pregnancies or previous births ($ps > .05$).

T-tests using relationship status (married vs unmarried) as the independent variable and background characteristics (age, number of previous pregnancies, and number of previous births) as dependent variables indicated that married women were significantly older than unmarried women ($t(58) = 2.9, p < 0.01$), but did not differ in the number of previous pregnancies or births ($ps > .05$). Chi-square analyses indicated that women who were married differed significantly from women who were unmarried in the frequency of reported income category ($\chi^2 (5) = 25.36, p < 0.00$). Inspection of the per-cell chi-square values indicated that all 6 of those who reported income of $30,000 or greater were married. Married women also differed from unmarried women in the frequency of education categories reported, with married women being more likely to report higher education levels ($\chi^2 (5) = 21.62, p < 0.00$).
Associations Among Demographics, Psychosocial Variables, and Health Behaviors

Associations among demographics and psychosocial variables. Correlations between demographic variables (pre-pregnancy BMI, age, education, income) and psychosocial variables (CES-D, PSS, PSAQ, TENSE, ISEL, BFSS, FSS, and Subjective SES, and parental attitudes) are presented in Table 4. Pre-pregnancy BMI was not associated with any psychosocial variable ($p > .05$). However, a relationship between depressive symptoms and lower BMI approached significance ($r = -.21, p = .12$).

As age increased, pregnancy-specific anxiety and depressive symptoms decreased ($p < 0.05$). Women who reported greater education also reported less pregnancy-specific anxiety ($r = .027, p = .05$). Income predicted greater social support as measured by the ISEL ($r = .33, p < .05$; See Table 4).

Finally, t-tests revealed that as compared to women who were married, women who were unmarried reported significantly less support as measured by the ISEL ($t(59) = -2.8, p < .01$). However, married and unmarried women did not differ in reports of support from the baby’s father (BFSS, $t(59) = -.45, p = .65$).

Associations among demographics and health behaviors. Next, analyses were conducted to examine relationships among demographic variables (BMI, age, income, and education) and health behaviors (exercise, prenatal vitamin use, sleep, and smoking). Specifically, t-tests were used to examine effects of exercise (regular versus not), prenatal vitamin use (daily versus not), and smoking status (current smoker versus not). These health behaviors did not predict differences in pre-pregnancy BMI, age, education, or income ($p > .05$). Further, correlational analyses demonstrated that there was no
relationship between the number of hours of sleep in the past night or past 3 nights on the day of the baseline visit with pre-pregnancy BMI, age, education, or income, \((p < 0.05)\).

In sum, analyses demonstrated that there were no significant associations between demographic variables and health behaviors.

**Associations among stress, depression, and health behaviors.** Analyses were then conducted to determine if stress or depressive symptoms were related to health behaviors. Unexpectedly, those who reported engaging in regular vigorous physical activity \((n=24)\) had significantly higher CES-D scores than those reporting that they did not engage in regular vigorous activity \((n=28)\), \(t(50) = -2.07, p = .04\). Women reporting regular exercise had an average score of 20 (SD = 10.4) on the CES-D while women reporting no regular exercise has an average score of 14 (SD = 10.0). This could reflect the fact that women who have more physically demanding jobs experience greater distress. To test this hypothesis, among the 26 women who reported that they were employed, their jobs were classified as those which likely involve much standing or other physical movement (e.g., housekeeping, fast food, warehouse worker, cashier) versus those which are likely more sedentary (e.g., customer service, telephone sales). When classified in this manner, 13 were classified as having active jobs and 12 as having inactive jobs while 1 did not report her type of employment. Twenty-eight women were unemployed.

Analyses were conducted to determine if women who were classified as having active employment tended to endorse that they engaged in regular vigorous physical activity. Among the 13 women who were classified as having physically active jobs, 1 did not complete the question regarding physical activity. Among the 12 who completed
This question, only 6 (50%) endorsed regular physical activity. Further, among those classified as having active employment, those who endorsed regular vigorous physical activity had an average score of 16 on the CES-D as compared to an average score of 12 among women who did not endorse regular vigorous physical activity. This difference was not statistically significant \((t(10) = -.69, p = .50)\). However, this was a small sample, and the direction of the effect is consistent with the hypothesis that those who perceive their jobs as involving more vigorous physical activity may experience greater distress.

Next, analyses were conducted to determine if women who were classified as having active employment reported a greater number of hours of vigorous activity per week. Two women were excluded from analyses of the number of reported hours of vigorous activity because their reports, 30 hours (unemployed) and 50 hours (active employment), were greater than 3 standard deviations from the mean. Means (standard deviations) for the reported number of hours of vigorous activity per week were as follows: active employment = 3.5 (4.6) hours, inactive employment = 1 (1.5) hours, unemployed = 2.2 (3.3) hours. One way-ANOVA analyses indicated no statistically significant difference in reported hours of vigorous active among those classified as having active employment, inactive employment, or unemployed \((F(2,49) = 1.7, p = .20)\). Post-hoc contrasts indicated that a difference between those in physically active versus inactive jobs approached significance \((p = .07)\).

In sum, the unexpected higher levels in CES-D scores among those endorsing regular vigorous physical activity likely reflect that a) some women were including work-related activity in their assessment of their activity levels while others were not and b)
those with jobs which they perceived to be physically demanding demonstrated a trend towards reporting greater depressive symptoms. Of note, analyses using classification of activity level of employment were preliminary based on estimates of which jobs were more versus less physically demanding. Women were not specifically asked about the extent to which their jobs involved standing, walking, lifting, or other physical activity. This type of assessment would be useful in future studies.

Women who reported regular prenatal vitamin use (5-7 days per week) did not differ significantly from those who reported no use in terms of depressive symptoms (t(57) = -1.8, p > .05) or perceived stress (t(57) = -.30, p > .05). Sleep in the past 24 hours or the past 3 days was not significantly associated with perceived stress or depressive symptoms (ps > .05). Finally, those who were current smokers at the baseline visit (n = 15) did not differ significantly from those who were not (n = 45) in perceived stress (t(58) = .82, p > .05) or depressive symptoms (t(58) = .90, p > .05).

Relationships among psychosocial factors. Finally, relationships among psychosocial factors were examined. As expected, depressive symptoms and perceived stress were positively correlated (r = .50, p < .001). Parental attitudes about pregnancy (i.e., maternal and perceived paternal happiness about pregnancy) were not significantly correlated with perceived stress or depressive symptoms (ps > .05). However, univariate ANOVA analyses demonstrated that women who were classified as unhappy about their pregnancies had significantly greater depressive symptoms (mean CES-D = 22, SD = 10) as compared to women who were classified as happy about their pregnancies (mean CES-D = 16, SD = 10; p = .04).
In terms of support, women who reported less social support as measured by the ISEL, Baby’s Father Support Scale, and/or Family Support Scale reported greater perceived stress, depressive symptoms, negative social interactions and significantly less maternal and paternal happiness about pregnancy \((ps < .05\); See Table 4). Moreover, frequency of both hostile and insensitive social interactions as measured by the TENSE were significantly related to depressive symptoms and perceived stress \((ps < .01\). Regression analyses demonstrated that after controlling for social support as measured by the ISEL, hostile social interactions remained a significant predictor of depressed mood \((\beta = .32, t(1,59) = 2.67, p = .01\). When included in a model together, social support as measured by the ISEL and negative social interactions as measured by the TENSE hostility subscale accounted for 42% of the variance in depressive symptoms. After controlling for social support as measured by the ISEL, insensitive social interactions were no longer predictive of depressed mood \((\beta = .17, t(1,59) = 1.25, p = .21\).

Further analyses were conducted to determine if perceived stress was a significant mediator of the relationship between social support and depressive symptoms. Regression analyses demonstrated that after accounting for the effects of perceived stress, social support as measured by the ISEL remained a significant predictor of CES-D scores \((\beta = -.44, t (2,55) = -3.66, p = .001\). Similarly, support from the baby’s father remained a significant predictor of depressive symptoms after accounting for perceived stress \((\beta = -.25, t (2,55) = -1.99, p = .05\) and the predictive value of family support was only marginally reduced \((\beta = -.19, t (2,55) = -1.5, p = .14\). In sum, overall the lower
depressive symptoms seen among those with greater support were not accounted for by
reductions in perceived stress.

Analyses were also conducted to determine if social support moderated the effect
of perceived stress on depressive symptoms. It was hypothesized that for among women
reporting high stress, those who also reported low support would be likely to report
greater depressive symptoms. Analyses using interaction terms (e.g., PSSXISEL),
revealed that the model violated the assumption of noncollinearity (VIFs > 10). Therefore,
the effects of these interaction terms were not interpretable.

**Associations Between Demographics, Health Behaviors, and Baseline IL-6**

Prior to testing hypotheses, IL-6 data were log transformed to normalize the data
distribution. Log-transformed data were used for all analyses; IL-6 refers to the log
transformed value for the remainder of analyses. Data points at or above 3 standard
deviations from the mean were considered to be outliers and were excluded from
analyses. After excluding outliers, data were available from 56 women at baseline and 33
women at baseline and follow-up.

Analyses were conducted to determine if IL-6 levels differed based on
demographic factors (age, race, marital status, education, employment status, income,
weeks gestation, trimester, parity, gravidity, and BMI). Age was not associated with IL-6
levels (r = -.07, p = .67). A relationship between race and IL-6 approached statistical
significance, t(49) = -1.45, p = .15, with African-American women exhibiting higher IL-6.
Specifically, mean IL-6 was 0.017 (SD = .20) lg pg/ml among Caucasian women and
0.135 (SD = .32) lg pg/ml among African-American women. There was no difference in IL-6 based on marital status (married versus unmarried), \( t(54) = 0.22, p = .83 \) or employment status (employed versus unemployed) \( t(54) = .46, p = .65 \). Correlational analyses demonstrated that IL-6 was not significantly related to gestational age at baseline \( (r = -.023, p = .87) \). Similarly, a t-test demonstrated that women in their first versus second trimester of pregnancy did not differ significantly in levels of IL-6, \( t(50) = .186, p = .85 \). IL-6 was not correlated with income, education, parity, or gravidity \( (p > .05) \). Pre-pregnancy BMI was significantly correlated with IL-6 \( (r = .54, p < .001) \). As described earlier, BMI was not significantly related with any other demographic characteristic, thus it was not used a covariate in the preceding analyses.

No health behavior (smoking, prenatal vitamin use, regular exercise, or sleep) was significantly associated with pre-pregnancy BMI. For this reason, analyses of associations between health behaviors and baseline IL-6 did not utilize BMI as a control variable. Results indicated no significant difference in IL-6 based on smoking status \( (t(54) = .125, p = .90) \), regular prenatal vitamin use \( (t(52) = -.99, p = .33) \), regular exercise \( (t(46) = -.02, p = .98) \), sleep in the past day \( (r = -.08, p = .58) \) or sleep in the past 3 days \( (r = -.04, p = .80) \). In sum, among demographic and health behavior variables, pre-pregnancy BMI was the only correlate of baseline IL-6 levels.

**Testing of Primary Hypotheses: Psychosocial Predictor of Inflammation**

*Associations Between Psychosocial Variables and Baseline IL-6.* Next, analyses were conducted to examine relationships between psychosocial factors and inflammation.
After excluding 2 outliers for which values were $\geq 3$ standard deviations from the mean, IL-6 samples were available at baseline from 56 women. Among these women, BMI was significantly negatively associated with CES-D scores ($r = -.31, p = .02$). An association between BMI and perceived stress scores also approached significance ($r = -.26, p = .06$). As described above, BMI was positively associated with baseline IL-6. Therefore pre-pregnancy BMI was used as a control variable in analyses related to depressive symptoms and perceived stress.

Analyses demonstrated that, after controlling for BMI prior to pregnancy, higher CES-D scores predicted significantly higher IL-6 ($\beta = .23, t (2, 55) = 1.98, p = .05$). Effects of depressive symptoms on baseline IL-6 are depicted in Table 5 and Figure 1. No other psychosocial variable (i.e., PSS, PSAQ, TENSE, BFSS, FSS, ISEL, Subjective SES, and parental attitude) was a significant predictor of baseline IL-6 ($ps > .05$).

**Associations Between Psychosocial Variables and IL-6 Responses Post-Vaccination.** Repeated measures analyses were conducted using baseline and follow-ups completed between 6-16 days post-vaccination ($n = 37$). In cases in which participants completed two visits during the specified time interval ($n = 8$), the average IL-6 value was utilized for analyses. Data points that exceeded 3 standard deviations of the mean were excluded from analyses. Therefore, final analyses included 33 women who provided data at both baseline and follow-up.

The predictive value of depressive symptoms and perceived stress were tested individually. In separate models, CES-D and PSS scores were used as continuous independent variables with IL-6 (repeated across time) as the dependent measure. First,
CES-D scores were utilized as the continuous independent variable (See Figure 2). Pre-pregnancy BMI was used as a control variable in these analyses. Results indicated no significant effect of time on change in IL-6 ($F(1,30)= .372, p = .55$). In addition, results indicated no significant interaction between time and depressive symptoms ($F(1,30)= 0.00, p = .95$). As in the baseline analyses, a significant main effect for depressive symptoms was seen ($F(1,30)= 6.6, p = .02$).

Also controlling for pre-pregnancy BMI, analyses using perceived stress scores as the independent variable indicated no significant effect of time on IL-6 levels ($F(1,31)= .12, p = .73$). There was also no significant interaction between PSS scores and change in IL-6 over time ($F(1,31)= 1.5, p = .23$) and no significant main effect for PSS scores ($F(1,31)= .00, p = .93$).

Finally, there were no significant relationships between any other psychosocial variable (i.e., PSS, PSAQ, TENSE, BFSS, FSS, ISEL, Subjective SES, and parental attitude) and change in IL-6 over time ($p_s > .05$)

**Moderating Effects of Social Support**

Next, analyses were conducted to examine the potential moderating effects of social support. Interaction terms were created using each of the three measures of social support (ISEL, BFSS, and FSS). Specifically, the following interaction terms were created: ISEL X PSS, BFSS X PSS, and FSS X PSS. Analyses revealed that relationships between these interaction terms with perceived stress and depressive symptoms violated
the assumption of noncolinearity (VIFs > 10). Therefore, moderating effects of social support could not be examined.

**Exploratory Analyses of Perinatal Outcomes**

Analyses were conducted to explore associations among psychosocial factors, inflammation, and three perinatal outcomes: maternal blood pressure, gestational age at delivery, and birth weight. Data from 2 women with twin pregnancies were excluded since multifetal gestation affects these perinatal outcomes.

**Birth Weight.** Infant birth weight data were available for 52 women. Low birth weight was defined as weight ≤ 2500 grams. Using this criteria, 7 women (13%) had low birth weight babies. Of these, 6 were also born preterm (i.e., prior to 37 weeks gestation). T-test analyses were performed to compare those with low versus normal birth weight babies. Analyses indicated that those with low birth weight babies reported significantly greater depressive symptoms (t(49) = -2.2, p = .03). Specifically, the average CES-D score among women with low birth weight babies was 25 (SD = 8) as compared to an average score of 16 (SD = 11) among those delivering normal birth weight babies. Further chi-square analyses indicated that women delivering low birth weight babies were significantly more likely to report depressive symptoms above a clinical cut-off of 16 on the CES-D (χ²(1,51) = 4.13, p = .04). In fact, 6 of the 7 women who delivered low birth weight babies were at or above a clinical cut-off for depressive symptoms. Chi-square analyses demonstrated that women with low birth weight babies differed significantly from those with normal birth weight babies in their likelihood of reporting
that they have been told they were clinically depressed ($\chi^2(1, 51) = 6.3, p = .03$). Four of 7 (57%) women who delivered low birth weight babies endorsed the question “Have you ever been told that you are clinically depressed” as compared to 7 of 44 (16%) of those with normal birth weight babies.

Further analyses demonstrated that those with low birth weight babies reported significantly greater hostile social interactions as measured by the TENSE ($t(49) = -2.34, p = .02$). Low birth weight was also associated with significantly higher scores on the item assessing feeling “concerned” on the pregnancy-specific anxiety questionnaire ($t(47) = -3.4, p < .01$).

Women with low birth weight babies also reported that they were significantly less happy about their pregnancies than were women with normal birth weight babies ($t(49) = 2.09, p = .04$). Among women with low birth weight babies, 1 was classified as happy, 3 were ambivalent, and 3 were unhappy. Thus, overall, 22% of women who were classified as either ambivalent or unhappy had low birth weight babies (6 of 27) while 4% of women reporting they were happy had low birth weight babies (1 of 25).

In terms of demographic factors, women with low versus normal birth weight babies did not differ in age ($t(49) = .45, p = .66$). All 7 of those who delivered low birth weight babies were unmarried. Three were African-American, 1 was Caucasian, and 3 reported mixed ethnicity. In terms of health behaviors, those with low birth weight babies did not differ from those with normal birth weight babies in the number of hours of sleep reported at their baseline visit, exercise, or prenatal vitamin use ($p > .05$). Those with low birth weight babies were significantly more likely to smoke ($\chi^2(1,51) = 4.28, p$
Specifically, 4 of 7 (57%) of those with low birth weight babies endorsed smoking during pregnancy as compared to 9 of 45 (20%) of those who delivered normal birth weight babies. Binary logistic regression analyses demonstrated that CES-D scores significantly predicted risk of low birth weight after controlling for smoking status (Exp(β) = 1.1, p = .02).

Women delivering low weight babies also demonstrated a trend toward having lower pre-pregnancy BMI than those delivering normal weight babies (t(46) = 1.8, p = .08). Logistic regression analyses demonstrated that after controlling for pre-pregnancy BMI, CES-D scores were still predictive of likelihood of having a low birth weight baby (Exp(β) = 1.1, p = .04). Thus, the effects of depressive symptoms on low birth weight were not mediated by low maternal weight prior to pregnancy.

Further analyses examined whether women delivering low birth weight babies differed significantly from those delivering normal birth weight babies in IL-6 levels at baseline. T-tests indicated a trend towards women with low birth weight babies to exhibit lower IL-6 (t(46) = 1.6, p = .12). A univariate ANOVA demonstrated that after controlling for pre-pregnancy BMI, there was not a significant difference in IL-6 between those who delivered low weight versus normal weight babies (F(1, 45) = .434, p = .51). Finally, women who delivered low birth weight babies did not differ from those delivering normal birth weight babies in their IL-6 responses to vaccination (F(1,28) = .94, p = .35).

**Preterm Delivery.** Next, analyses were conducted to examine relationships among psychosocial factors, inflammatory markers and preterm delivery. After excluding 2
women with multifetal gestation and 1 woman who developed preeclampsia during her pregnancy, data regarding gestational age at delivery were available from 52 women. These women were excluded from analyses related to gestational age at delivery because women with multifetal gestation or preeclampsia are more likely to deliver preterm.

Among the 52 women included in these analyses, a total of 7 (13%) women delivered preterm. Of these, 6 were also low birth weight. Analyses revealed that a relationship between preterm delivery and depressive symptoms approached statistical significance (t(49) = -1.6, p = .12). Further chi-square analyses found no statistically significant difference in the frequency of women scoring above a clinical cut-off for depressive symptoms (χ²(1,51) = 1.4, p = .24). However, it is notable that 5 of the 7 women who delivered preterm scored at or above a clinical cut-off for depressive symptoms. In addition, chi-square analyses demonstrated that women who delivered preterm were significantly more likely to report that they had previously been told that they were clinically depressed (χ²(1,51) = 6.1, p = .01).

In terms of demographic factors, women who delivered preterm versus full-term did not differ in age (t(49) = -.32, p = .75). Six of 7 of those who delivered preterm babies were unmarried. Two were African-American, 2 were Caucasian, and 3 reported mixed ethnicity. In terms of health behaviors, those who delivered preterm did not differ from those who delivered at full-term in the number of hours of sleep reported at their baseline visit, exercise, or prenatal vitamin use (p > .05). Those with preterm babies were significantly more likely to be current smokers (χ²(1,51) = 5.10, p = .02). Specifically, 4
of 7 (57%) of those who delivered preterm endorsed smoking during pregnancy as compared to 8 of 45 (18%) of those who delivered full-term babies.

T-test analyses indicated that a relationship between preterm delivery and IL-6 approached statistical significance, with those delivery preterm exhibiting lower IL-6 (t(46) = 1.9, \(p = .06\)). However, there was also a trend for women who delivered preterm to have lower pre-pregnancy BMI (t(46) = 1.5, \(p = .13\)). Univariate ANOVA analyses indicated that after controlling for pre-pregnancy BMI, women who delivered preterm exhibited no significant difference as compared to those who delivered full-term in levels of IL-6 (F(1, 45) = 1.7, \(p = .22\)).

**Blood pressure.** Finally, analyses were conducted to examine relationships among psychosocial factors, IL-6, and blood pressure during pregnancy. In the current investigation, only 1 woman met diagnostic criteria for preeclampsia. For this reason, blood pressure data were examined continuously rather than being dichotomized. Data from the one woman with preeclampsia was excluded from these analyses because her blood pressure values deviated significantly from the mean. In addition, one woman was excluded because her highest systolic blood pressure measurement of 140 mmHg was at the level of gestational hypertension and was greater than 3 standard deviations from the mean. Thus, these data points were excluded because their inclusion would skew analyses examining blood pressure data in a continuous fashion. Of note, data from these 2 women were included in analyses related to inflammatory markers; their IL-6 levels did not deviate significantly from the mean and the participant who developed preeclampsia did not have this condition at the time of her study participation. Finally, data from 2 women
with twin pregnancies were excluded because multi-fetal gestation affects blood pressure. Therefore, the effects of psychosocial factors on cardiovascular function may differ during singleton versus multi-fetal pregnancies. After these participants were excluded, blood pressure data were available from 52 participants.

First, correlational analyses were conducted to examine relationships between the highest systolic and diastolic blood pressures recorded at regular perinatal visits and psychosocial variables. Unexpectedly, results indicated a significant negative association between the highest systolic blood pressure during pregnancy and depressive symptoms \((r = -.43, p = .002)\), perceived stress \((r = -.28, p = .04)\), pregnancy-specific anxiety \((r = -.37, p = .009)\), the TENSE insensitivity scale \((r = -.43, p = .002)\), and the TENSE hostility scale \((r = -.29, p = .04)\). In addition, there was a positive relationship between support from the baby’s father and highest systolic blood pressure \((r = .28, p = .05)\).

In terms of diastolic blood pressure, a negative relationship between depressive symptoms approached significance \((r = -.25, p = .08)\). In addition, a significant negative relationship was seen between the highest diastolic blood pressure and the TENSE insensitivity scale \((r = -.29, p = .03)\).

Next, regression analyses were conducted in which age and pre-pregnancy BMI were used as covariates. Results indicated that after controlling for these covariates, highest systolic blood pressure remained significantly associated with each of the previously described predictors: depressive symptoms \((t(3,51) = -3.05, \beta = -.43, p = .004)\), perceived stress \((t(3,51) = -2.0, \beta = -.27, p = .05)\), pregnancy-specific anxiety \((t(3,48) = -2.6, \beta = -.38, p = .01)\), TENSE insensitivity scores \((t(3,51) = -3.5, \beta = -.46, p = .001)\),
TENSE hostility scores \( t(3,50) = -2.09, \beta = -.29, p = .04 \), and support from the baby’s father \( t(3,51) = 2.2, \beta = .30, p = .03 \).

After controlling for pre-pregnancy BMI and age, the relationship between highest diastolic blood pressure and depressive symptoms was reduced slightly \( t(3,51) = -1.7, \beta = -1.7, p = .09 \). The negative relationship between highest diastolic blood pressure and TENSE insensitivity scale \( t(3,51) = -2.3, \beta = -.30, p = .03 \) remained significant.

Because effects of lower blood pressure were seen, additional analyses were conducted using cut-offs for low blood pressure. Low systolic blood pressure was defined as maximum systolic blood pressure of 110 mmHg. Of the 52 women for whom blood pressure data were available, 12 (23\%) met this criteria. Low diastolic blood pressure was defined as a maximum diastolic blood pressure of 60 mmHg during pregnancy. Eight women (15\%) met this criteria. These cut-offs were chosen based on previous research on hypotension during pregnancy (e.g., Friedman & Neff, 1978; Ng & Walters, 1992).

Univariate ANOVA analyses were conducted using age and pre-pregnancy BMI as covariates. Results demonstrated that in comparison to those with higher systolic blood pressures, those with maximum systolic blood pressure of 110 or lower reported greater perceived stress \( F(1,51) = 4.7, p = .04 \), greater depressive symptoms \( F(1,51) = 4.5, p = .04 \), and greater incidence of insensitive interactions \( F(1,51) = 6.8, p = .01 \).

Additional univariate ANOVA analyses demonstrated that in comparison to those with higher diastolic blood pressures, those with a maximum diastolic blood pressure of 60 or lower reported greater depressive symptoms \( F(1,50) = 8.05, p = .007 \), greater incidence of insensitive interactions \( F(1,51) = 5.7, p = .02 \), and greater incidence of
hostile interactions ($t(49) = 5.3, p = .03$). Thus, results from analyses in which blood pressure data were analyses continuously versus dichotomously were highly consistent.

Regression analyses demonstrated no relationship between highest systolic or diastolic blood pressure during pregnancy and IL-6 at the baseline visit ($ps > .05$). Similarly, univariate ANOVAs revealed no significant differences in IL-6 between those classified as having low versus high systolic or diastolic blood pressures ($ps > .05$).

Finally, analyses were conducted to determine whether low blood pressure predicted risk of low birth weight or preterm delivery. T-tests demonstrated that those who delivered pre-term did not differ significantly in their highest systolic blood pressure ($t(47) = 1.4, p = .17$) or diastolic blood pressure ($t(47) = .78, p = .44$). Similarly, women delivering low birth weight versus normal birth weight babies did not differ significantly in their highest systolic ($t(47) =1.3, p = .20$) or diastolic blood pressure ($t(47) = 1.8, p = .29$).
A good deal of research links 1) stress and negative perinatal outcomes, 2) inflammation and negative perinatal outcomes and 3) stress and inflammation in non-pregnant populations. However, only a few published studies to date have attempted to link stress and inflammation during pregnancy. The extent to which data from non-pregnant samples generalizes to pregnancy is unknown because there are substantial changes in immune function during pregnancy. Moreover, no studies have linked psychosocial factors, inflammation, and perinatal outcomes within the same model.

The current study was designed to address gaps in the current literature by examining direct and priming effects of stress and depression on inflammatory markers during pregnancy. In order to examine priming effects, influenza vaccination was used as an antigen challenge. Accordingly, data were collected pre and post-vaccination to allow for assessment of direct as well as priming effects of stress/depression on inflammatory parameters. In addition, data related to maternal health (i.e., blood pressure) and birth outcomes (i.e., low birth weight, preterm delivery) were collected to allow for preliminary analyses linking stress, inflammation, and perinatal outcomes within a single model.
Demographic and Psychosocial Characteristics of the Sample

In the current sample, the majority of women were unmarried (77%), African-American (57%), and from lower socioeconomic backgrounds (86% reported total family income < $30,000 per year). In concert with their demographic characteristics, women reported high levels of depressive symptoms and perceived stress. In fact, 52% of women scored at or above a clinical cut-off for depressive symptoms as measured by the Center for Epidemiologic Studies Depression scale (CES-D). In comparison, in the general population, only 10-20% of pregnant women would be expected to meet criteria for clinical depression (Gotlib et al., 1989; Marcus et al., 2003; O'Hara et al., 1984).

The high rates of depressive symptoms seen in the current sample are similar to other studies of pregnant women from lower socioeconomic backgrounds. For example, Seguin and colleagues (1995) found that 47% of pregnant women whose incomes were below the poverty line scored at or above a clinical cut-off for depressive symptoms as compared to 20% of pregnant women from higher socioeconomic backgrounds. These and other studies highlight the prevalence of clinically significant depressive symptoms among women of low socioeconomic status.

Similarly, the average score on the Perceived Stress Scale (PSS) was 6.65 (SD = 2.82) on a scale of 0-15. In comparison, the average perceived stress score is estimated to be 4.7 (SD = 3.1) among women overall in the general population (S. Cohen &
Williamson, 1988). Thus, the high levels of perceived stress among women in this sample are also notable.

In terms of social support, the average score on the ISEL was 38.75 in the current sample. In population-based samples, mean scores range from approximately 33-34 points overall, with women tending to score somewhat higher than men (S. Cohen et al., 1985). Therefore, scores on the ISEL were in the range typically seen in the women in the general population. In addition, scores on the baby’s father support scale (BFSS) and Family Support Scale (FSS) were similar to previous studies. As described, in the current sample, the average score on the BFSS was 3.23 (SD = .91) and the average score on the FSS was 3.32 (SD = .74). In comparison, in a study of over 200 pregnant women, the average score on the BFSS was 3.45 (SD = .47) while the average score on the FSS was 3.33 (SD = .43) (Feldman et al., 2000). It is surprising that scores on the BFSS are not lower in the current sample, as the sample in the study by Feldman et al., 67% of participants were married. It is also notable that there were not significant differences in perceived support from the baby’s father based on marital status in the current sample (t(59) = -.45, p = .65). This is unexpected, as previous research has shown that young partners of unwed mothers tend to provide limited economic and social support (Rangarajan & Gleason, 1998). However, married women did report greater overall support than did unmarried women as measured by the ISEL.

Social support was highly predictive of both depressive symptoms and perceived stress. In validation studies of the ISEL, correlations between the ISEL and CES-D ranged from -.52 to -.60 (Cohen et al., 1985). Therefore, the correlation of -.54 seen in
the current sample is typical. These data support the hypothesis that social support is a key source of coping and suggest that women lacking adequate support may be especially vulnerable to feeling overwhelmed and experiencing depressive symptoms during pregnancy.

As predicted, negative social interactions as measured by the Test of Negative Social Exchange (TENSE) were significant predictors of both depressive symptoms and perceived stress. Moreover, after accounting for the effects of social support as measured by the ISEL, hostile social interactions remained a significant predictor of depressive symptoms. Together, social support and hostile social interactions accounted for 42% of the variance in depressive symptoms. In sum, these data are consistent with previous data demonstrating that social support and social conflict are unique and robust predictors of risk of depressive symptoms (Westdahl et al., 2007).

Analyses were conducted to examine potential mediating and moderating effects of social support. In terms of mediating effects, it was predicted that perceived stress would mediate the relationship between social support and depressive symptoms. However, analyses indicated that social support was still predictive of depressive symptoms after controlling for perceived stress. Therefore, this hypothesis was not supported. It is possible that such mediating effects would be seen in a prospective design in which social support and perceived stress were used to predict the development of future depressive symptoms rather than concurrent depressive symptoms.

It was also hypothesized that social support would moderate the effects of perceived stress on depressive symptoms. That is, it was predicted that lower social
support would be more strongly predictive of higher depressive symptoms among individuals reporting high stress. However, analyses using interacting terms (e.g., PSS X ISEL) demonstrated that these variables violated the assumption of non-collinearity with other variables in the model, therefore these results could not be interpreted.

In the current sample, 47% of women reported that they were ambivalent or unhappy about their pregnancy. In addition, 38% reported that the father of the baby was ambivalent or unhappy about the pregnancy. Notably, women who reported that they were unhappy about their pregnancies (n = 12) had significantly greater depressive symptoms than did women reporting that they were happy about their pregnancies (n = 33). Although direction of causality between depressive symptoms and happiness about pregnancy can not be determined in the current study, these data are consistent with the contention that pregnancy itself can be a significant source of distress, particularly pregnancies that are unplanned or unwanted. Moreover, maternal and paternal happiness about pregnancy have predicted birth weight in previous research (Keeley et al., 2004). Thus, the high degree of ambivalence and unhappiness about pregnancy is notable.

**IL-6 Across Pregnancy**

As described, IL-6 was not associated with week of gestation. This is not unexpected; IL-6 changes are typically not seen across pregnancy in healthy women, except for soon before delivery. For example, in a study of women who developed preeclampsia during pregnancy as compared to controls, those who developed preeclampsia exhibited a 77% increase in IL-6 from 1st to 3rd trimester while control
participants exhibited no significant increase in IL-6 (Freeman et al., 2004). In addition, it is of note that a limited number of women (n = 5) were in their third trimester of pregnancy. Also, among those who were in the second trimester (i.e., 13-26 weeks), the majority were in the earlier stages (median = 16.8 weeks). Therefore, the ability to track changes across pregnancy was somewhat limited. Finally, the current analyses focused only on one marker. Changes across pregnancy, as well as interactions between weeks gestation and stress in predicting inflammation, may have been evidenced with other inflammatory markers.

Associations Among Demographic Variables, Health Behaviors, and IL-6

In the current sample, IL-6 levels were positively correlated with body mass index (BMI). This is expected, as IL-6 is produced by adipocytes (fat cells; Vilcek, 2003). In addition, depressive symptoms predicted significantly lower pre-pregnancy BMI. This is consistent with the fact that change in appetite, either increased or decreased, is a frequent depressive symptom (Basco et al., 1997; Radloff, 1977). Relatedly, major depression may be marked by significant weight loss or significant weight gain. For example, in a study of 109 clinically depressed outpatients, 30% demonstrated significant weight loss, 40% significant weight gain, and 30% demonstrated no weight change during their current depressive episode (Weissenburger, Rush, Giles, & Stunkard, 1986). As compared to other symptoms of depression (e.g., cognitive symptoms), changes in weight and appetite are more commonly seen in more severe cases of depression (Simon & Von Korff, 2006). Moreover, it has been forwarded that weight loss is a sign of more
severe depressive symptoms, while weight gain is associated with milder depressive symptoms (Paykel, 1977; Weissenburger et al., 1986). Thus, the association between lower BMI with depressive symptoms may reflect that the overall severity of depressive symptoms in the current sample was high. Of note, the association between lower BMI with greater depressive symptoms in the current sample is consistent with evidence that depressive symptoms predict poorer weight gain during pregnancy (Bonari et al., 2004; Orr et al., 2002; Zuckerman et al., 1989).

Low SES has been associated with higher levels of inflammatory markers (e.g., Koster et al., 2006; Steptoe, Owen, Kunz-Ebrecht, & Mohamed-Ali, 2002). This effect was not seen in the current study; income, education, and subjective SES were not associated with IL-6 levels. Relatedly, income, education, and subjective SES were not significantly predictive of perceived stress or depressive symptoms in the current sample. Lack of differences due to socioeconomic status may be attributable to the restricted range of incomes represented. As described 86% of the women reported a total family income of less than $30,000 per year. In addition, 82% reported that their highest level of education was high school or less. Consequently, the ability to detect effects of socioeconomic status was limited due to this range restriction.

In addition to socioeconomic status, potential differences based on race were of interest. Notably, African-Americans tend to exhibit elevations in inflammatory markers as compared to Caucasians (e.g., Kiecolt-Glaser et al., 2003). One factor contributing to higher IL-6 among African-Americans is a significantly greater prevalence of genetic polymorphisms which promote production of IL-6 (Mulherin Engel et al., 2005; Simhan,
Krohn, Roberts, Zeevi, & Caritis, 2003). Chronic stress associated with minority status may further promote elevations in IL-6 among African-Americans, particularly among those exhibiting genetic vulnerability. Thus, it has been proposed that, via inflammatory pathways, both genetic and environmental factors likely contribute to the 3 to 4-fold greater risk of preterm delivery seen in African-American as compared to Caucasian women (e.g., Giscombe & Lobel, 2005; Goldenberg, Culhane, Iams, & Romero, 2008; Mulherin Engel et al., 2005; Simhan et al., 2003). In the current investigation, IL-6 levels did not differ statistically between African-American and Caucasian women. However, African-American women exhibited a non-significantly higher level of IL-6. The ability to detect this effect may have been limited by the sample size. Power analyses indicated that the difference seen in the current sample was equivalent to an effect size of .44 in terms of Cohen’s $d$ which is a medium effect. To detect an effect of this size with .80 power at $\alpha = .05$, 130 women would be needed. Future research utilizing a larger sample size would help to better describe effects of race on inflammation during pregnancy.

**Primary Hypotheses: Psychosocial Predictors of Inflammation**

The primary hypotheses in the current study were that 1) stress and depressive symptoms would predict baseline IL-6 and 2) higher stress and/or depressive symptoms would predict greater inflammatory responses to influenza vaccination.

In the current investigation, depressive symptoms were significantly related to baseline IL-6, with greater depressive symptoms predicting higher IL-6. These results are comparable to data from non-pregnant populations demonstrating that depressive
symptoms predict higher levels of circulating inflammatory markers, including IL-6 (Maes et al., 1995; Penninx et al., 2003; Zorrilla et al., 2001). Notably, the current study provides the first data linking depressive symptoms to circulating levels of inflammatory markers during pregnancy. These data support the contention that depressive symptoms may contribute to negative perinatal outcomes, including preterm delivery, via inflammatory pathways.

Unexpectedly, perceived stress was not significantly predictive of IL-6 in the current sample. This is contrary to previous data demonstrating that stress predicts higher IL-6 during pregnancy (Coussons-Read et al., 2007; Coussons-Read et al., 2005). Differences in the predictive value of perceived stress in the current sample versus previous studies may be attributable to sample characteristics as well as differences in methods of measurement. First, the women in the current study were generally from significantly lower socioeconomic backgrounds than women in previous studies. For example, in the sample studied by Coussons-Read et al. (2007), participants’ average education was 14.6 years and the average annual income was $46,000. Accordingly, the lack of effect of stress in the current sample may reflect that under conditions of sufficient stress, the experience of depressive symptoms is a better predictor of physiological effects than is perceived stress. In addition to marked differences in sample characteristics, the measure of perceived stress used in the current study differed from that used by Coussons-Read et al. (2005 & 2007) which used the Denver Maternal Health Assessment (DMHA, Meikle, Orleans, Leff, Shain, & Gibbs, 1995). Therefore, it is
possible that differences in how stress was measured contributed to lack of effect of stress in the current study.

In terms of response to vaccination, no significant change in IL-6 was seen over time. In addition, no interactions were found between IL-6 response to vaccination and psychosocial factors. Thus, the hypothesis that those reporting greater stress or depressive symptoms would evidence greater IL-6 responses to vaccination was not supported.

Timing of the post-vaccination assessment is one key factor that may have contributed to a lack of effect of vaccination on IL-6 overall as well as lack of differences in IL-6 response based on stress or depressive symptoms. Indeed, one study of inflammatory responses to influenza vaccination showed that IL-6 increased at 1 day post-vaccination and returned to baseline levels by 3 days post-vaccination (Tsai et al., 2005). Similarly, Posthouwer and colleagues (2004) demonstrated that influenza vaccination causes transient increases in C-reactive protein (CRP) that peak at 2 days post-vaccination. As described above, data from Glaser and colleagues (2003) demonstrated that depressive symptoms predicted IL-6 responses at 2 weeks post-vaccination. Given data from other samples, effects seen at 2 weeks are likely to represent dysregulated prolonged inflammatory responses to vaccination. Accordingly, measurement at 1-2 days post-vaccination as well as 1-2 weeks post-vaccination would allow for assessment of both immediate as well as prolonged responses.

Another consideration is that pregnancy likely affects the extent to which IL-6 responses occur in response to vaccination. Indeed, rats injected with lipopolysaccaride (LPS) exhibit increases in both TNF-α and IL-6 when not pregnant. However, during
pregnancy, rats respond to LPS with increases in TNF-α but not IL-6 (Fofie et al., 2004). Thus, lack of an overall IL-6 response may be a reflection of normal adaptation that occurs during pregnancy. Again, measurement at multiple timepoints post-vaccination would allow for this question to be addressed. In addition, utilization of a non-pregnant control group of equivalent age and socioeconomic background would allow for examination of which effects are pregnancy-specific.

Finally, age is an important moderator of inflammation, with age tending to exacerbate inflammatory responses to immune challenge (Graham, Christian, & Kiecolt-Glaser, 2006c). The women in the current study were significantly younger than the older adult samples utilized in previous studies which have reported effects of stress or depressive symptoms on IL-6 responses to influenza vaccination. The average age of participants in the study by Glaser and colleagues (2003) which demonstrated that depressive symptoms predict greater IL-6 responses to influenza vaccination was 71 years (SD = 9). Similarly, a study by Segerstrom and colleagues (2008) demonstrated that individuals experiencing the chronic stress of caregiving demonstrated greater IL-6 responses to influenza vaccination than did control subjects at 4 weeks post-vaccination. The average age of their participants was 75 years (SD = 7). As described, the average age in the current sample was 25 years (SD = 5). Accordingly, lack of IL-6 response to vaccination among participants in the current sample may be attributable to age.
Exploratory Analyses of Perinatal Outcomes

To provide data for development of future studies, exploratory hypotheses related to three perinatal outcomes were examined. Outcomes of interest were the following: 1) birth weight, 2) gestational age at delivery, and 3) maternal blood pressure. Each of these outcomes has been linked to stress and depression during pregnancy. The goal of the current analyses was to explore whether preliminary evidence supported a link between stress and perinatal outcomes within the current sample and, if so, whether inflammation may mediate this link.

Preterm Birth and Low Birth Weight. Results indicated that those who delivered low birth weight babies had significantly greater depressive symptoms than those delivering normal birth weight babies. Indeed, 6 of 7 of the women who delivered low birth weight babies were at or above a clinical cut-off for depressive symptoms. Although women scoring above a clinical cut-off for depressive symptoms were more likely to smoke and have lower BMI prior to pregnancy, these factors did not appear to mediate the association between depressive symptoms and low birth weight. Women who delivered low birth weight babies also reported greater frequency of hostile social interactions and less happiness about being pregnant than did their counterparts who delivered normal birth weight babies.

Similarly, a trend for those delivering preterm having higher depressive symptoms approached significance. Specifically, those delivering preterm had mean CES-D score of 23 (SD = 8.8) while those delivering at term had a mean score of 16.6 (SD = 10.7). Power analyses indicated that this is equivalent to an effect size of .66 which
is a medium to large effect. In order to detect an effect of this size with .80 power at $\alpha = .05$, 120 participants would be needed to detect this effect if the ratio of preterm to term deliveries (i.e., 7/45) remained constant.

There was a high degree of overlap between low birth weight and preterm delivery. In the current sample, 6 out of 7 of the low birth weight babies were also preterm while 6 out of 7 of the preterm babies were also low birth weight. Consequently, distinguishing separate effects of low birth weight versus preterm delivery was not possible. Although an overall effect for depressive symptoms was suggested, there was not a significant relationship between preterm delivery or low birth weight and IL-6. In sum, preliminary results support the hypothesis that depressive symptoms measured relatively early in pregnancy are predictive of preterm delivery and/or low birth weight. However, differences in IL-6 were not implicated in this link.

**Blood Pressure.** In terms of blood pressure, only 1 woman met criteria for preeclampsia during her pregnancy and 1 had possible gestational hypertension. Therefore, analyses relating stress to high blood pressure during pregnancy were not possible. However, analyses unexpectedly revealed significant relationships between low blood pressure during pregnancy and greater depressive symptoms, stress, and negative social interactions. These relationships remained after controlling for body mass index prior to pregnancy and age.

There appear to be no studies to-date examining associations between low blood pressure and mood during pregnancy. However, at least 4 studies have described associations between low blood pressure and depression in elderly populations (Barrett-
Connor & Palinkas, 1994; Jorm, 2001; Paterniti, Verdier-Tailefer, Geneste, & al., 2000; Stroup-Benham, Markides, Black, & al., 2000). For example, in a study of 846 men ages 60-89 years, low diastolic blood pressure (< 75 mmHg) was associated with significantly higher depressive symptoms (Barrett-Connor et al., 1994). This relationship was independent of age or weight loss. Similar effects have been reported across age groups. In a population-based study of over 60,000 men and women ages 20-89 years, both low systolic and diastolic blood pressure (≤ 5th percentile) were associated with greater anxiety and depression (Hildrum et al., 2007). These relationships remained after controlling for numerous potential confounds including age, sex, health conditions, use of drugs for hypertension, body mass index, and smoking status.

It has been suggested that the relationship between low blood pressure and depressive symptoms may reflect increased fatigue among those with low blood pressure (Barrett-Connor et al., 1994; Tonkin, 2004). However, within the current sample, analyses of individual CES-D items predicting low blood pressure indicated that those items that were most predictive of blood pressure were not related to fatigue. Specifically, the following items were most predictive of low blood pressure: I felt depressed (r = -.42, p = .002), I felt lonely (r = -.34, p = .015), and I thought my life had been a failure (r = -.48, p = .002). There is no item on the CES-D that specifically inquires about fatigue. Blood pressure was marginally associated with an item that likely taps into fatigue, “I could not get going” (r = -.25, p = .07). However, overall, the current association between depressive symptoms and low blood pressure did not appear to be attributable to fatigue.
Low blood pressure was not predictive of low birth weight or preterm delivery in the current sample; however power to detect possible effects was low. Although gestational hypertension has been the focus of much research, limited attention has been focused on the potential effects of low blood pressure during pregnancy. In 1961, McClure Browne reported that in a prospective study of 7,344 pregnancies, a U-shaped curve was seen in terms of the relationship between blood pressure and perinatal outcome; women with very low systolic blood pressure (less than 105 mmHg) or very high systolic blood pressure (greater than 140 mmHg) showed increased risk of perinatal death. Subsequently, Friedman and Neff (1978) reported that low systolic and diastolic blood pressure predicted greater risk of low birth weight and fetal death as well as lower intelligence scores at age 4 in offspring (Friedman et al., 1978).

Since these early findings (Friedman et al., 1978; McClure Browne, 1961), few follow-up studies have been conducted to examine hypotension during pregnancy. Goeschen and colleagues reported that maternal hypotension predicted greater risk of fetal distress, delivery by C-section, low birth weight, and perinatal mortality (Goeschen, Pluta, Meyer-Wilmes, & Saling, 1982). Similarly, in a study of 596 hypotensive versus 596 normotensive women, those classified as hypotensive experienced greater incidence of premature birth, intrauterine growth retardation, low birth weight, and increased perinatal mortality (Harsanyi & Kiss, 1985). Articles by both Goeschen (1982) and Harsanyi (1985) were published in German, although abstracts are available in English. In terms of research published in English, Marguiles found that among Latin American women, diastolic blood pressure chronically below 65mmHg or above 85mmHg was
predictive of low birth weight (Margulies et al., 1987). Also, Ng and Walters (Ng et al., 1992) found that women who were chronically hypotensive (blood pressure \(\geq 110/70\) mmHg) had shorter length of gestation, their babies were smaller for gestational age, and they were more likely to suffer from postpartum complications.

There has been significant controversy as to whether such findings reflect low blood pressure as a causal risk factor versus a correlate of other risk factors (e.g., low BMI, low SES). It has been argued that studies reporting links between low blood pressure and negative perinatal outcomes have not adequately measured or controlled for possible confounding variables. In a relatively recent study, Zhang and Klebanoff (2001) reported that in a sample of 28,095 pregnancies, low blood pressure was associated with preterm birth and small for gestational age babies. However, they also found that women with lower blood pressure also tended to be younger, shorter, lighter, leaner, poorer, more likely to be minority, and likely to gain less weight during pregnancy. After controlling for these factors, low blood pressure was not associated with negative perinatal outcomes. They concluded that, in general, low blood pressure does not increase the risk of negative perinatal outcomes. However, they also noted that low blood pressure due to specific factors (e.g., compromised plasma volume expansion or pathological homeostasis) may be an exception.

Limited research has examined mechanisms by which low blood pressure may cause negative perinatal outcomes. Two studies (published in German with English abstracts) have demonstrated that hypotension predicts reduced uteroplacental blood flow (Gronberger, Leodolter, & Parschalk, 1979; Scheler & Ropke, 1993). Adequate placental
perfusion is critical to fetal health. Therefore, this represents one pathway by which maternal hypotension may negatively affect perinatal outcomes.

In sum, the current data indicate that depressive symptoms, as well as stress and hostile social interactions are predictive of low blood pressure after controlling for age and body mass index. There is limited evidence linking low blood pressure and depressive symptoms in the general population. There is also mixed evidence linking low blood pressure to negative birth outcomes. However, to-date, the extent to which associations between maternal hypotension and negative outcomes are attributable to confounding variables is debatable. Results from the current study do not support the contention that low blood pressure causes increased scores on measures of depression due to fatigue. However, a plausible pathway by which depressive symptoms may cause low blood pressure has not been identified. It is possible that a third factor (e.g., diet, genetics) that was not identified or measured in the current study accounts for both low blood pressure and increased depressive symptoms.

Limitations

As described above, one important limitation of the current study is that only a single post-vaccination follow-up assessment was conducted. As a result, the full inflammatory response trajectory over time could not be determined. Future research utilizing multiple timepoints would allow for better descriptions of typical and dysregulated immune responses to vaccination and other types of antigen challenge during pregnancy.
Another limitation of this study is that the majority of participants were in their 1st or early 2nd trimesters. A larger sample size with equivalent groups across each trimester would be ideal for assessing potential change in inflammatory markers and inflammatory responses across pregnancy. In addition, a non-pregnant comparison group would provide much information regarding changes that occur during pregnancy.

The current analyses focused only on IL-6 outcomes. There are clearly a variety of other neuroendocrine and immune markers including other inflammatory cytokines, antiinflammatory cytokines, hormones, and catecholamines that have important implications for mood, health, and perinatal outcomes. Thus, analyses of additional markers would allow for more a comprehensive picture of psychophysiology during pregnancy.

This study utilized the CES-D as a measure of depressive symptoms. As described, this measure has excellent reliability, validity, and has predictive value for important perinatal outcomes. As noted, the CES-D does not allow for diagnosis of clinical depression. However, as in the current investigation, depressive symptoms are predictive of physical health outcomes including immune function (Glaser et al., 2003) and mortality (Steptoe, Wardle, & Marmot, 2005). Moreover, the assessment of depressive symptoms via a questionnaire measure provides an inexpensive and efficient method for screening and identifying women who may benefit from further assessment and intervention in a clinical setting. Future research in which diagnostic clinical interviews are utilized in conjunction with questionnaire measures would allow for better
determination of the predictive validity as well as clinical utility of assessing depressive symptoms versus clinical depression.

Finally, the current study primarily included women of lower SES. As described, women in the study reported high levels of stress and depressive symptoms highlighting the relevance of questions related to psychosocial correlates of perinatal health for this population. However, the extent to which these findings translate to other populations is not known. In addition, in the current sample, women reporting low levels of stress were underrepresented. The ability to detect effects of stress and depressive symptoms would be enhanced in a sample which included a broader range of scores on these psychosocial measures.

Implications

The current study provides the first data linking depressive symptoms to inflammation during pregnancy. These data are consistent with the hypothesis that one pathway by which depressive symptoms may affect perinatal outcomes is via dysregulation of inflammatory processes. Thus, this is an important factor which may contribute to notable differences in risk of negative perinatal outcomes based on race and socioeconomic status.

The current study highlights the importance of assessing depressive symptoms during pregnancy. Within the current sample, stress and clinically significant depressive symptoms were highly prevalent. Moreover, lack of social support was a strong predictor of greater perceived stress and depressive symptoms. Assessment of post-partum
depression is common and has been recommended as a standard of care (Gjerdingen & Yawn, 2007; Seehusen, Baldwin, Runkle, & Clark, 2005). However, assessment of depressive symptoms during pregnancy has received significantly less attention (Lee et al., 2007). Data from this and previous studies clearly demonstrate that depressive symptoms during pregnancy are predictive of physical health, behavior, and perinatal outcomes, arguing for the importance of addressing depressive symptoms during pregnancy as well as postpartum.

In addition to assessment of depressive symptoms during pregnancy, the provision of appropriate interventions is necessary. The high rates of depressive symptoms in the current population emphasize the need for greater information regarding pharmacological, behavioral, and alternative treatments for depression during pregnancy. Key to such future efforts will be weighing the risks and benefits of medication against the potential harms of untreated depression during pregnancy.

**Future Directions**

The current investigation focused on responses to a physical challenge of vaccination during pregnancy. Also of interest is whether individual differences in responses to acute psychological stressors may affect perinatal outcomes. Evidence to-date indicates that cardiovascular responses to acute stressors are attenuated during pregnancy (de Weerth & Buitelaar, 2005) although little is known about potential moderators of this effect (e.g., depressive symptoms, SES). Moreover, limited research to date has examined neuroendocrine or immune responses to stress during pregnancy and
no studies to-date have examined associations between cardiovascular, neuroendocrine, and immune responses during pregnancy within a single study (de Weerth et al., 2005). This represents an area in which greater investigation is needed.

The current study provided preliminary data regarding perinatal outcomes. Future studies may target populations at-risk for low birth weight, preterm delivery, hypertension, or hypotension. Oversampling women who are likely to experience such negative outcomes would improve power to detect effects of psychosocial factors on these outcomes. Moreover, in addition to examination of maternal and birth outcomes, future research should aim to establish links between prenatal development with mental and physical health across the lifespan.

Finally, a primary goal of research in this area should be the development and implementation of safe and effective interventions targeting depression as well as the physiological and behavioral sequelae of depression. Available data regarding the safety of antidepressant medication use during pregnancy is not sufficient to allow women to make an informed choice regarding this treatment strategy (Bonari et al., 2004). In addition, in order to avoid potential harmful consequences of medication during pregnancy, behavioral approaches (e.g., cognitive-behavioral therapy, exercise) are highly appropriate for pregnant populations. Finally, alternative treatment strategies, particularly supplementation with omega-3 fatty acids, hold great promise. Accumulating evidence indicates that low levels of essential fatty acids contribute to risk of depression via inflammatory pathways (Kiecolt-Glaser et al., 2007). Moreover, low levels of omega-3 fatty acids are predictive of increased risk for preterm delivery (e.g., Reece, McGregor,
Allen, & Harris, 1997) and preeclampsia (e.g., Williams, Zingheim, King, & Zebelman, 1995). Thus, omega-3 supplementation is an intervention strategy which may have multifaceted benefits for perinatal health.

In sum, links among psychosocial factors, physiological factors, and perinatal health outcomes have only begun to be delineated. The fetal period is a critical period in human development. In addition, pregnancy is a time of significant change which can be mentally and physically challenging for the mother. Psychosocial factors which affect maternal physiology likely contribute to disparities in maternal and fetal health outcomes based on race and socioeconomic status. Therefore, delineating and addressing effects of psychosocial factors on mental and physical health during pregnancy is critical for optimizing health during pregnancy and throughout the lifespan.
REFERENCES


Maes, M., Ombelet, W., De Jongh, R., Kenis, G., & Bosmans, E. (2001). The inflammatory response following delivery is amplified in women who previously suffered from major depression, suggesting that major depression is accompanied by a sensitization of the inflammatory response system. *Journal of Affective Disorders, 63*, 85-92.


APPENDIX A

TABLES
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Description</th>
</tr>
</thead>
</table>
| Age (years)                            | Range: 18-37  
Mean: 25.24 (SD = 4.89) |
| Race                                   | African-American = 34  
Caucasian = 19  
Asian = 1  
Bi-racial = 6 |
| Marital Status                         | Unmarried = 46  
Married = 14 |
| Education                              | Less than high school = 19  
High school = 30  
Greater than high school = 11 |
| Employment Status                      | Employed = 25  
Unemployed = 35 |
| Income                                 | < $15,000 = 38  
15,000-29,999 = 14  
>30,000 = 6 |
| Body Mass Index (kg/m²)                | Pre-pregnancy: 27.78 (SD = 6.08)  
At baseline visit: 28.54 (SD = 6.72) |
| Weeks Gestation at Baseline            | 15 (SD = 7.8) |
| Trimester at Baseline                  | 1st trimester = 31  
2nd trimester = 24  
3rd trimester = 5 |
| Parity                                 | 2.1 (SD = 1.8) |
| Gravidity                              | 1.4 (SD = 1.2) |

Table 1: Demographic Characteristics
<table>
<thead>
<tr>
<th>Measure</th>
<th>Range</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CES-D</td>
<td>1-41</td>
<td>17.05 (10.14)</td>
</tr>
<tr>
<td>PSS</td>
<td>0-13</td>
<td>6.65 (2.82)</td>
</tr>
<tr>
<td>PSAQ</td>
<td>4-18</td>
<td>10.37 (3.96)</td>
</tr>
<tr>
<td>TENSE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hostility</td>
<td>0-3</td>
<td>0.65 (0.73)</td>
</tr>
<tr>
<td>Insensitivity</td>
<td>0-3</td>
<td>0.78 (0.95)</td>
</tr>
<tr>
<td>ISEL</td>
<td>16-48</td>
<td>38.75 (7.25)</td>
</tr>
<tr>
<td>BFSS</td>
<td>8-32</td>
<td>3.23 (0.91)</td>
</tr>
<tr>
<td>FSS</td>
<td>7-28</td>
<td>3.32 (0.74)</td>
</tr>
<tr>
<td>Parental Attitudes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother</td>
<td>1-10</td>
<td>6.93 (3.13)</td>
</tr>
<tr>
<td>Father</td>
<td>1-10</td>
<td>7.87 (2.59)</td>
</tr>
<tr>
<td>Subjective SES</td>
<td>1-8</td>
<td>4.7 (1.7)</td>
</tr>
</tbody>
</table>

**Table 2: Scores on Psychosocial Measures**
<table>
<thead>
<tr>
<th>Health Behavior</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current Smoker</td>
<td>15</td>
<td>45</td>
</tr>
<tr>
<td>Prenatal Vitamin Use</td>
<td>Daily: 43</td>
<td>1-5 days per week: 3</td>
</tr>
<tr>
<td>Regular Exercise</td>
<td>Yes: 24</td>
<td>No: 28</td>
</tr>
<tr>
<td>Sleep</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hours (past day)</td>
<td>6.65 (SD = 1.8)</td>
<td></td>
</tr>
<tr>
<td>Hours (past 3 days)</td>
<td>21.10 (SD = 6.66)</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3: Health Behaviors**
Table 4: Product-moment correlations among demographic and psychosocial characteristics. * $p < .05$, ** $p < .01$.

Abbreviations: BMI = Pre-pregnancy Body Mass Index; Sub SES = Subjective SES; PA Mother = Maternal Parental Attitudes; PA Father = Paternal Parental Attitudes
### Baseline IL-6

<table>
<thead>
<tr>
<th>Step</th>
<th>Predictor</th>
<th>Estimate $\beta$</th>
<th>$\Delta R^2$</th>
<th>$\Delta F$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pre-pregnancy BMI</td>
<td>.610</td>
<td>.29</td>
<td>22.14</td>
<td>&lt; .01</td>
</tr>
<tr>
<td>2</td>
<td>CES-D</td>
<td>.232</td>
<td>.05</td>
<td>3.93</td>
<td>.05</td>
</tr>
</tbody>
</table>

Table 5: Regression Analyses - Relationship between depressive symptoms and baseline IL-6
**Figure 1:** IL-6 levels prior to vaccination after controlling for Body Mass Index prior to pregnancy. Results are depicted here using a clinical cut-off of ≥ 16 for illustration purposes. Regression analyses controlling for BMI prior to pregnancy and utilizing CES-D scores as a continuous measure demonstrated that depressive symptoms predicted significantly higher IL-6 prior to vaccination ($p = .05$).
Figure 2: IL-6 levels pre and post-vaccination after controlling for Body Mass Index prior to pregnancy. Results are depicted. Results are depicted here using a clinical cut-off of $\geq 16$ for illustration purposes. Repeated measures general linear model analyses utilized CES-D scores as a continuous measure. Among the 33 women for whom data were available at both baseline and post-vaccination, no significant change in IL-6 was seen over time. A significant main effect for depressive symptoms was evidenced, with greater depressive symptoms predicting higher IL-6 ($p < .05$).